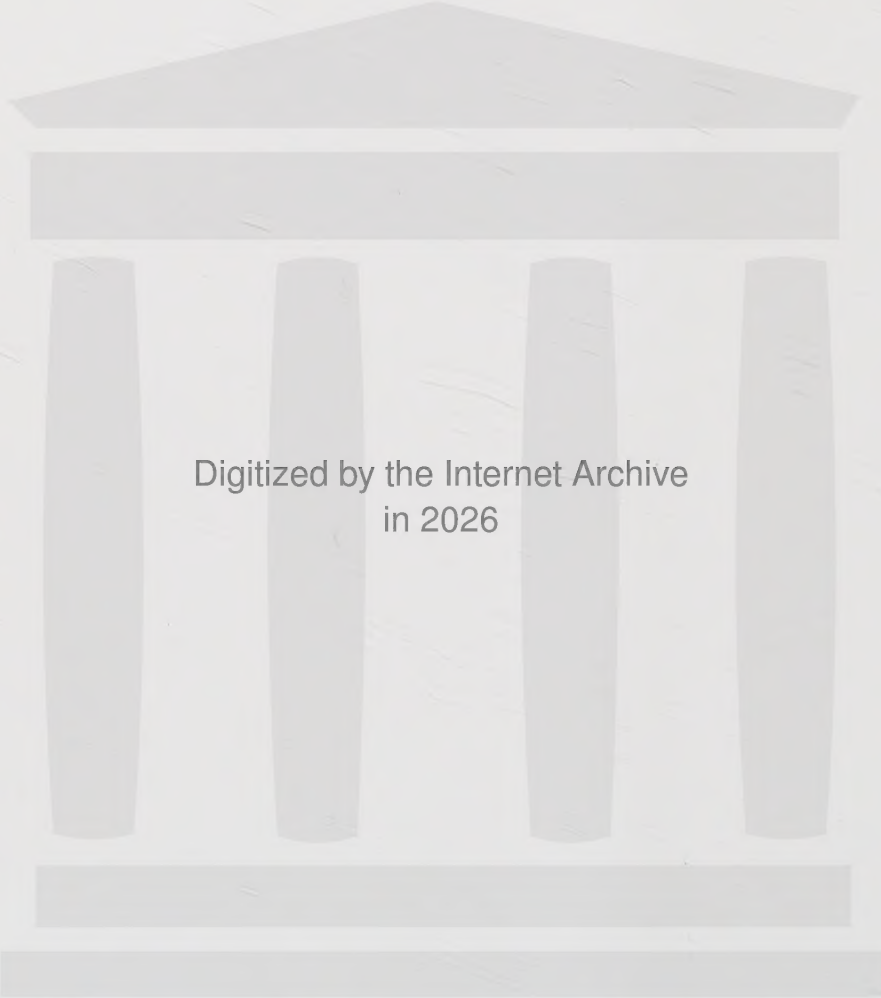


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THE ANATOMICAL RECORD

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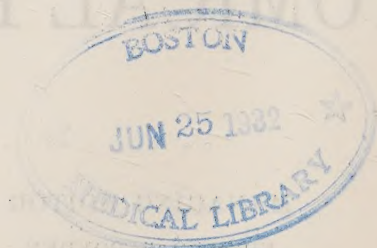
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THE CYTOLOGICAL PROCESSES INVOLVED IN THE FORMATION OF THE SCALAE OF THE INTERNAL EAR

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TWO PLATES (TWENTY-THREE FIGURES)

Recent experimental work on the development of the auditory apparatus has given an impetus to the study of the formation of the internal ear. One of the most interesting approaches to this problem is that of Fell ('28) in which normal parts of the fowl ear were allowed to develop in vitro, using explants of the otocyst. As a result of this work, Fell is of the opinion that any possible influences exercised by the nervous system, vascular system, or associated organs may be eliminated. This would seem to indicate that the influences which control the development of the various parts of the internal ear emanate alone from the ectodermal anlage.

However, before positive conclusions can be reached from experimental work of this kind, all the normal developmental processes must be thoroughly understood. Among the most interesting and the least comprehended portions of the ear are the periotic spaces. Very little work has been done on the detail of the process of origin of these cavities. The only pertinent article with which the writer is familiar is that of Streeter ('17) dealing with the development of the scalae of the human embryo. He found that these spaces which are hollow and lined with flat epithelium in the adult develop from compact mesenchyma by an enlargement and coalescence of existing mesenchymal spaces. As so aptly pointed out by this author, the formation of these normal

spaces or scalae is not a capricious phenomenon, but is a precise and orderly process of development. Although Streeter was not primarily concerned with the cytological features of their development, he is of the opinion that, in the human embryo, the enlargement and coalescence of spaces during the formation of the scalae is essentially a process of retraction and rearrangement of the mesenchymal elements and that this tissue is not lost in the process, but actively participates in it.

The present investigation was undertaken in the hope of throwing more light on the nature of these processes and particularly of ascertaining whether or not the mesenchymal syncytium is used up in the formation of the scalae. The writer is indebted to Doctor Hardesty not only for suggesting this problem, but also for his most generous assistance.

MATERIALS AND METHODS

Some forty embryos and fetuses ranging from 2 to 14 cm. (crown-rump) were sectioned for study. The adult cochlea was also used for making measurements. In some cases the whole embryo or fetus was fixed after the brain had been removed from the cranium. In such specimens measurements were made following fixation. In other instances measurements were made first; after which the head was sectioned sagittally, the brain removed from the cranium, and the two halves of the head were submerged in Held's fluid. This latter method gave slightly better fixation.

Both the paraffin and celloidin methods of embedding were employed. The results obtained by the use of the paraffin method were practically worthless because of the shrinkage; accordingly, this method was discarded in favor of the celloidin technique. In those fetuses in which the cartilaginous (prebony) capsule was present better preparations were obtained by leaving some of the tissue around the capsule, for the pressure that was sufficient to clear away all the pericapsular tissue seemed to injure the softer internal tissues. A dissection laying bare the cochlea previous to embedding was not attempted in specimens under 5 cm.

Considerable difficulty was experienced in securing a satisfactory cytological stain. After much experimenting with various formulae, the following technique was developed. Celloidin sections 13 to 15 μ in thickness were mordanted from three to six hours in a modified Benda's solution. This was prepared by taking 25 cc. of Benda's mordant (Lee, '28) and adding 70 cc. of 95 per cent alcohol. Following mordanting, the sections were washed thoroughly in 70 per cent alcohol and stained for twelve to twenty-four hours in a 0.5 per cent solution of hematoxylin in 70 per cent alcohol. The only object in using alcoholic solutions was to avoid unnecessary injury from passage of sections through the low grades of alcohol to water and then back through the higher alcohols. After the stained sections were differentiated in the mordant, thoroughly washed in 70 per cent alcohol, and passed into 95 per cent alcohol, they were counterstained in a 0.5 per cent solution of erythrosin in beechwood creosote. Then the excess erythrosin was removed by rinsing in pure creosote and the sections were passed into oil of cedar wood, from which they were mounted in balsam. This technique gives an excellent differentiation of nuclear and cytoplasmic constituents. The celloidin is not colored by the mordant and the erythrosin persists as a permanent and brilliant dye in the syncytial cytoplasm, the finer processes of which are very delicate and usually invisible with other techniques.

ORDER OF APPEARANCE OF THE PERIOTIC TISSUE SPACES

In the human embryo (Streeter, '17) the periotic cistern of the vestibule develops first, then the scala tympani, and finally the scala vestibuli. But in the pig embryo, as observed in these studies, the scala vestibuli develops before the scala tympani. The first space to appear, however, is the vestibular cistern, which is just visible in 4-cm. embryos. By the 4.5-cm. stage it is well developed. Thus the process of space formation, beginning in the vestibule, progresses from vestibule to cochlea and in the cochlea from base to apex.

In 6-cm. fetuses the first indication of the scala vestibuli may be seen in axial sections of the basal whorl of the cochlea. Almost immediately afterward, the scala tympani becomes visible (fig. 18). As space formation progresses from base to apex, the scala vestibuli of a given whorl always keeps definitely in advance of the corresponding tympani (see fig. 21, an axial section of an 8.5-cm. fetus).

THE CYTOLOGY OF THE DEVELOPING SCALAE

Fetuses of 8.5 cm. are most favorable for this study. In specimens of this length the scalae have not appeared in the most apical whorl. Coils basal to it show all gradations in their development. The scalae begin their formation on the sides of the spiral ganglion. In these localities the undifferentiated periotic reticulum is composed in the early stages of close-meshed, delicate strands of cytoplasm (figs. 1, 22). The first indication of space formation, as also reported by Streeter for the human embryo, is an enlargement of the meshes of the reticulum (A, fig. 2). This is followed by attenuation, parting, and retraction of the delicate mesenchymal strands. At this early period the retraction of the strands is the only observable feature. But as development proceeds a new process appears which is destined to become more and more conspicuous. This is the detachment and subsequent degeneration of individual mesenchymal cells. The cytoplasm of such cells undergoes granular degeneration and the nucleus usually undergoes karyolysis. In some instances retrograde changes in the nucleus are of a pycnotic character (fig. 10). There is the possibility that the retraction phenomenon, associated with liquefaction, is in itself the initial step in degeneration, as suggested by the recently shown moving pictures of rat sarcoma, in which degeneration following irradiation is preceded by the withdrawal and rearrangement of the cytoplasmic processes.¹

¹In considering the part that liquefaction plays in this process, it is instructive to recall the work of Thomson ('19) on the formation of the fluid of the human graafian follicle. He asserts that it is formed by disintegration and liquefaction (plasmorrhaxis) of the granulosa cells, liquefaction being preceded by granula-

Once the spaces have developed to the size of those shown in *B*, figure 2, evidence of degeneration is of frequent occurrence. Two or more such spaces then unite to form a larger one, following degeneration of a portion of the limiting wall. Cellular degeneration is characterized, first, by granulation of the cytoplasm (figs. 4, 9, 14, 19, 20, 23) and then by karyolysis (figs. 4, 5, 15, 17, 23). The latter is often preceded by a massing of the chromatin to one side of the nucleus (figs. 4, 14, 17).

In the later phases of space formation the process of degeneration is characterized by the appearance of cells that have been interpreted as connective-tissue macrophages (figs. 6, 7, 11, 12, 23). The cytoplasm of these cells is highly vacuolated and often contains one or more ingested, degenerating nuclei (figs. 7, 12). Figures 6 and 11 show cells of this type which are devoid of ingested nuclei, but which contain a granular vacuolated cytoplasm. The evidence thus indicates that the products of degeneration, other than those which may undergo liquefaction, are removed by phagocytes.

As development proceeds, larger and larger spaces coalesce, following granular degeneration of the syncytial connecting strands, until the scalae are completed. For a while stiff, blunt granular processes may be seen projecting into the spaces (fig. 16). These later undergo degeneration (figs. 5, 17). As the process continues a limiting wall may undergo degeneration at more than one locus, resulting in the detachment of a portion of the reticulum. Most of these fragments immediately degenerate, but occasionally they round up into plasmodia (fig. 13), the ultimate fate of which has not been

tion of the cytoplasm. In a similar way disintegration of the cytoplasm of the periotic mesenchyma presumably results in accumulation of fluid and withdrawal of connecting strands. The fact that the macrophages contain very little granular material in their vacuoles suggests that much of the degenerating cytoplasm undergoes liquefaction. Once the accumulation of fluid is started, further enlargement of the spaces might proceed by pressure atrophy as well as by plasmorrhaxis. Somewhat more problematical is the extent to which infiltration of lymph from surrounding tissues may aid in the enlargement of the periotic spaces. Certainly it is difficult to understand how such infiltration, if it occurs at all, could initiate the process of space formation.

determined. Presumably these masses degenerate with liquefaction or are ingested by phagocytes.

The epithelium which eventually forms the outer limit of the scalae is derived from the cells separating the most peripheral spaces from the unmodified mesenchyma. Before the cells are transformed into the epithelium they are characterized by rounded nuclei and an open-meshed cytoplasm which exhibits processes projecting into the space (similar to those of *B*, fig. 2). With the enlargement of the scalae the nuclei of the border cells become elongated and flattened and the connecting cytoplasmic strands are pressed back into a continuous border which is smooth on the side next to the cavity (fig. 8). Apparently the epithelium thus formed is similar to the mesenchymal epithelium that lines the cavities of joints and bursae.

DISCUSSION

From a consideration of the above facts it is evident that the chief problem in the development of the scalae is the cause of the initial changes in the periotic mesenchyma. Since it was suggested that the early separations of cytoplasmic processes might be due to mechanical stresses in the growth of the whole cochlea, careful measurements of sections of this organ were made throughout the entire period of development. The figures obtained from fourteen specimens, so measured, showed that at the 6- and 7-cm. stages—the period in which the initial formation of the scalae takes place—there was less growth in the main basal and axial planes of the cochlea than at any subsequent or preceding period. Although the relatively small number of specimens used is insufficient for statistical study, the preliminary findings suggest that growth stresses play an insignificant rôle in the early formation of the periotic spaces.

The most significant finding in this investigation, therefore, seems to be the occurrence of degeneration at the site of the formation of the scalae and the simultaneous appearance of connective-tissue macrophages. Such participation

of macrophages in the removal of degenerating tissues is of common occurrence in even younger stages of vertebrate embryos. Boyden ('22, '18) was among the first to point out their rôle in the removal of vestiges of gill filaments of chick embryos, and the modeling and attrition of such areas as the anal plate, caudal intestine, and cloaca. The difficult thing in these instances, as in the periotic spaces, is to determine what causes the initial degeneration of epithelial or mesenchymal tissues in well-vascularized portions of the embryo.

This consideration, together with the results obtained by recent experimental work, suggests that the factors that control the development of the mesodermal components of the inner ear reside in the otic vesicle itself. Ogawa ('26) has shown that the cartilaginous capsule of the ear in amphibians does not form following removal of the otic vesicle. Filatow ('27) states that following transplanting of the otic vesicle of amphibians to regions of undifferentiated mesenchyma the otic vesicle stimulates the mesenchymal cells to the formation of a new capsule. The results of Fell's work on the development of the isolated otocyst in vitro have already been mentioned. Altogether, these various findings would therefore seem to indicate that some influence, emanating from the otic vesicle, controls the development of the various parts of the inner ear and presumably initiates the formation of the periotic spaces.

SUMMARY

Development of the scalae in pig embryos begins in the basal turn of the cochlea and progresses toward the apex. Contrary to the conditions in the human embryo, the scala vestibuli begins in advance of the scala tympani, and continues to maintain its lead.

Cytologically, the first step in the formation of the scalae is an enlargement of the mesenchymal spaces. Cavities, thus initiated, coalesce to form still larger ones. The initial process seems to consist of liquefaction accompanying attenua-

tion, separation, and retraction of the mesenchymal strands, but this is soon followed by visible degeneration of these strands and the removal of granular débris by connective-tissue macrophages. The scalae thus enlarge through destruction of the mesenchyma, and not merely by a rearrangement of that tissue.

Measurements of the growing cochlea show that during the period in which the initial formation of the scalae takes place there is less growth in the main axial and basal planes than at any subsequent or preceding period. Therefore, it is believed that mechanical growth stresses play an insignificant, if any, part in the early formation of the periotic spaces. Indeed, the evidence obtained by other authors from experimental removal of the otocyst suggests that the degenerative changes that occur at the site of the periotic spaces and that initiate the formation of the scalae—as described in this article—arise in response to factors which reside in the membranous labyrinth.

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PLATES

All the figures of plate 1 were drawn at table level with a camera lucida. The figures of plate 2 are photomicrographs of selected areas from the developing scalae of 8.5-cm. pig fetuses.

PLATE 1

EXPLANATION OF FIGURES

- 1 Area of undifferentiated mesenchyma.
- 2 An early stage in the development of a scala. Note the cytoplasmic portion of a macrophage in space *B*. The nucleus was not sectioned.
- 3 Cytoplasmic degeneration of a portion of the periotic reticulum. The nucleus is as yet apparently unaffected.
- 4 A portion of the wall of a space, showing cytoplasmic degeneration and beginning nuclear dissolution. Note the massing of the chromatin to one side of the nucleus. The nuclear membrane also appears to be thinner in this region.
- 5 A projecting portion of the reticulum, showing cytoplasmic and nuclear degeneration. One nucleus is apparently normal, but with no surrounding cytoplasm, whereas the other is undergoing disintegration. In this mass are many isolated, stiff-appearing portions of cytoplasm which have resisted degeneration. Not all of the granular material shown is the product of cytoplasmic granulation; much of it is coagulum of the periotic fluid.
- 6 and 7 Drawings of two macrophages. Figure 7 shows a macrophage which has ingested two nuclei—one, pyknotic and enclosed in a vacuole; the other, staining very lightly.
- 8 A portion of the wall of a scala which is almost completed, illustrating the character of the epithelium.

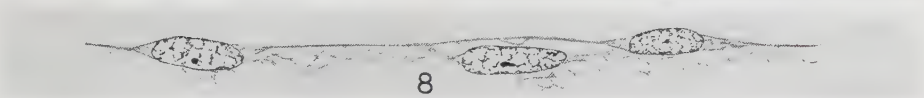
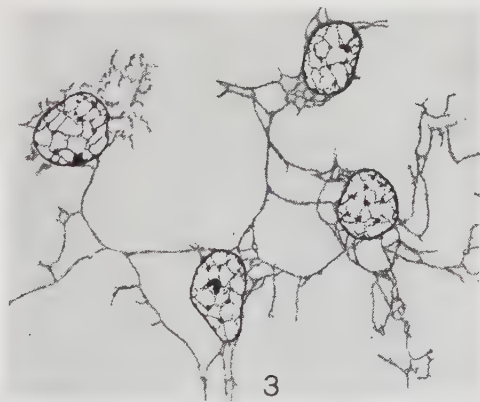
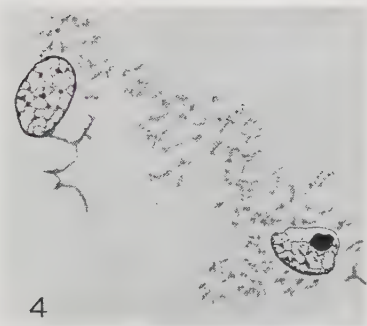
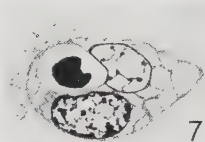
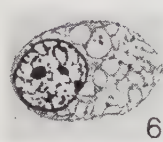
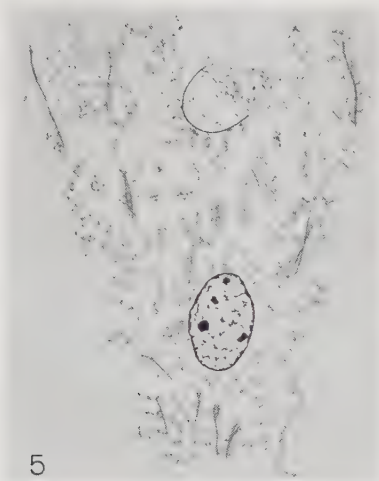
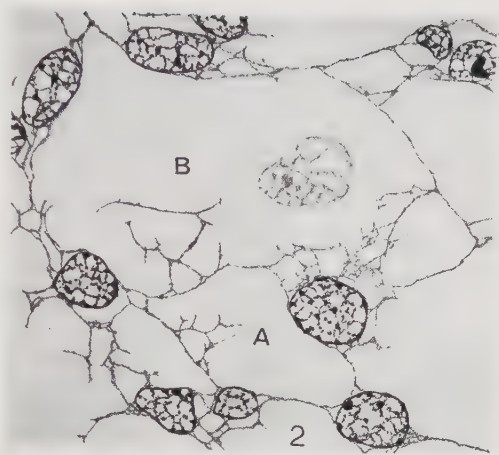
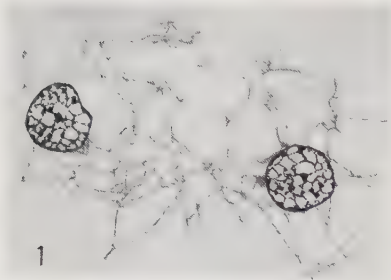


PLATE 2

EXPLANATION OF FIGURES

9 Granular degeneration of the cytoplasm.

10 A portion of the syncytium undergoing degeneration by pyknosis and granulation of the cytoplasm.

11 and 12 Photomicrographs of macrophages shown in figures 6 and 7 of plate 1.

13 A syncytial mass which became detached during the coalescence of spaces and later rounded up.

14 and 15 Nuclei undergoing dissolution. Attention is directed to the massing of the chromatin in figure 14.

16 A portion of the peripheral wall of a nearly completed scala. Note that only local areas in the wall have broken down, leaving processes of the syncytium projecting into the scala.

17 Nuclei undergoing dissolution. Note rupture of one of the nuclear membranes.

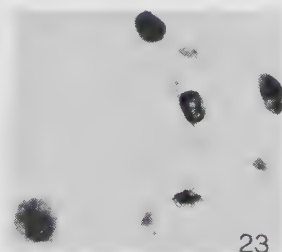
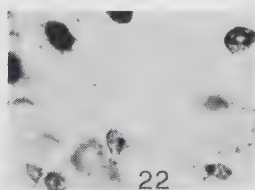
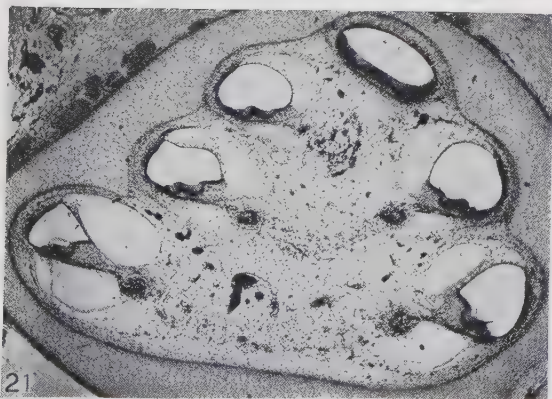
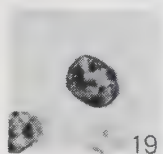
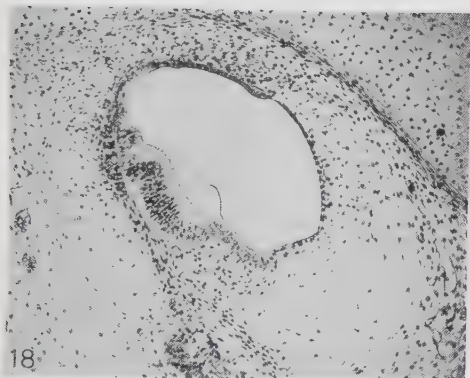
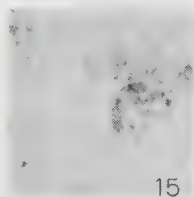
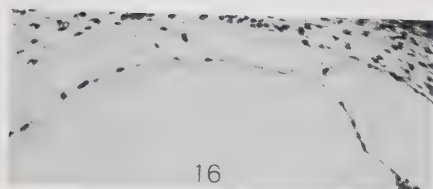
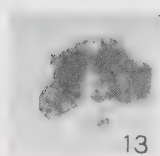
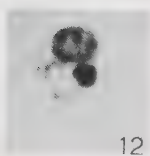
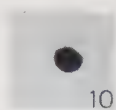
18 An apical coil which shows the scala vestibuli to be more advanced in its development than the scala tympani.

19 and 20 Granular degeneration of the cytoplasm.

21 Section of the cochlea of an 8.5-cm. fetus, showing the stages in the formation of the scalae.

22 An area of undifferentiated mesenchyma in a region of scala formation.

23 An area from a developing scala, showing degeneration of cytoplasm and nuclei. Note four degenerating nuclei. At the lower left-hand corner a macrophage may be observed.



STUDIES IN STAIN TECHNIQUE

III. A CYTOLOGICAL METHOD FOR STAINING WITH THE EHRlich-BIONDI MIXTURE

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The principal extracellular component of the testis and ovary is collagenous fibrous tissue. In cytological investigations of these organs and especially those dealing with the origin of the germ cells it is essential to differentiate the parenchyma from the surrounding tissue while retaining all cytological details. This contrast is not obtained with any of the more commonly used cytological stains. Although the Ehrlich-Biondi mixture has been virtually discarded in cytological procedures, it has the essential stains needed; the methyl green is a specific for chromatin, the acid fuchsin is used in certain techniques as a collagen stain (Mallory's acid fuchsin, Van Gieson's picric-acid fuchsin), and the orange G is an excellent cytoplasmic stain. Believing that the successful employment of this stain might depend upon special fixation or mordanting of the tissues—as has proved to be true of other techniques in which aniline dyes were employed (cases in which osmic acid was found to be the needed agent, e.g., Winiwarter, '23; Allen, '27; Foley, '29, '30¹)—the writer has undertaken to find out whether or not the Ehrlich-Biondi stain would react positively in tissues that had been fixed or mordanted in fluids containing osmic acid.

¹ Attention is directed to a typographical error in the actual per cent dye content of acid fuchsin as stated in this paper. It is given as 0.65 gram actual dye or 0.65 per cent aqueous solution of acid fuchsin, whereas it should read 0.065 gram or per cent.

TISSUES AND STAINS EMPLOYED

Testes and ovaries of the mud minnow (*Umbra limi*) and of the white mouse were used. Tissues were fixed in Allen's B15 and Flemming's strong fluid, the former containing no osmic acid, the latter the usual proportions of this reagent. Those fixed in B15 were mordanted, previous to staining, in a 0.5 per cent aqueous solution of osmic acid.

A methyl green, an acid fuchsin, and an orange G, dispensed by Coleman & Bell Co., were employed. The most constant results were obtained by using the following stock solutions:

Methyl green (49 per cent, representing 0.1127 gram actual dye), 0.23 gram per 100 cc. of distilled water.

Acid fuchsin (59 per cent, representing 0.0649 gram actual dye), 0.11 gram per 100 cc. of distilled water.

Orange G (86 per cent, representing 0.086 gram actual dye), 0.1 gram per 100 cc. of distilled water.

Prior to use, the solutions should be mixed in the following order: 10 cc. of glycerine, 20 cc. of acid fuchsin, 30 cc. of orange G; then slowly and with constant stirring, 80 cc. of methyl green.

PROCEDURE

The same general method that was devised for handling Auerbach's mixture (Foley, loc.cit.) may be employed. Following the removal of paraffin, the sections are passed through potassium permanganate and oxalic acid to remove substances left in the tissue by the fixative, and then thoroughly washed in running water. If the tissue has previously been fixed in a non-osmicated fluid, they are now mordanted in a 0.5 per cent aqueous solution of osmic acid. Again they are washed in tap-water or distilled water, and then stained for twelve to twenty-four hours in the previously described stain mixture. After staining, the excess stain is wiped from each slide and the sections lightly pressed by blotting-paper. The slides are now immersed, for five to thirty seconds, in 95 per cent alcohol that has been acidulated by one or two drops of hydrochloric acid (36 to 37 per cent). If one desires to study the chromosomes only, a longer time should be allowed for

destaining. Ten seconds is usually sufficient to bring out the details of resting nuclei. From acid alcohol the sections are transferred directly to carbol-xylol and then into xylol.

By this method excellent results are obtained with gonadal tissues. Chromatin that is compact stains light green; less densely arranged nuclear material, blue-green; collagen bundles, red, and cytoplasm, brownish yellow.

SUMMARY

Testicular and ovarian tissues can be made to stain with a modified Ehrlich-Biondi mixture if they are fixed in an osmicated fluid or mordanted in aqueous solutions of osmic acid. This stain is of value in cases in which it is desirable not only to differentiate sharply between the germ cells and the surrounding stroma, but also to secure finer cytological detail than is obtained by the use of the more commonly employed differentiating stains.

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OUR INADEQUATE TERMINOLOGY CONCERNING THE ANTERIOR PALATINE REGION

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The value of any name as a distinguishing device lies in its sole applicability to a particular thing. Its etymological suitability is a secondary matter. In anatomy, descriptive and associative terms usually are easiest for the student, as the terms mandible, mandibular foramen, canal, artery, and nerve illustrate.

All anatomists are aware of the great advantages of the Basle terminology, although some terms retained as a concession to long usage are undesirable. The term submaxillary gland is one of these and incisive foramen is another. The use of the latter term is particularly confusing, because there may be as many as six foramina in the region concerned.

I have long felt that American anatomists could perform a real service to anatomy if they took the initiative and came to an understanding among themselves and suggested the elimination of some of the objectionable terms that still remain in the Basle terminology. That, however, is a larger problem than the one that immediately concerns me here.

When I noticed the presence of cysts in the median inter-incisive region of the maxilla in association with the incisive canals, almost two decades ago, the defects in the terminology of this region became fully evident. Since I have maintained silence regarding these difficulties for almost two decades, I trust that no one will conclude that I ever have had any sympathy with the retrogressive suggestion to abandon the B N A. At the time this suggestion was made, I recall saying that if any anatomists wished to go backward instead of for-

ward, it was their privilege to do so, but that I saw no special reason why they should wish to stop at the eleventh edition of a certain work, for they might just as well go back to the very beginning of things, and there rest content in their accomplishment.

Since I did not care to include suggestions regarding terminology in a paper on "Hitherto unrecognized median maxillary cysts,"¹ I am making them independently here. Students of elementary gross anatomy who read their textbooks of systematic anatomy thoughtfully and consult their manuals, atlases, and dictionaries with a skull before them, always are perplexed regarding the present terminology in the anterior palatine region. Nor does the examination of a series of skulls help them out of their difficulties. In the Basle terminology only one incisive foramen and canal are recognized, but the skulls frequently reveal the presence of two or more canals, as Scarpa showed, which may open on a plane surface, or into an oval depression with a concave floor—which is a true fossa—or into a relatively wide, short canal.

The text in gross anatomy is likely to confirm the student's observation regarding the frequent presence of more than one incisive canal by referring to the canals of Stenson and of Scarpa. In about half of the skulls the student will not be able to find an opening in the anterior region of the palate, answering to what the books call the incisive foramen, but a fossa or canal, or from one to six foramina according to the work of Matiegka ('00) and Le Double ('06). Rarely, he will find no opening at all, and not infrequently he will see a shallow oval depression, in the floor of which one or more canals open, and which can be designated rightly as a fossa, but not as a foramen. Yet he finds that the only incisive fossae recognized by his text are located on the anterior surfaces of the jaws. Usually the oval fossa which he finds in the anterior region of the palate extends more or less obliquely in a craniocaudal plane, or lies almost in a vertical plane on the posterior surface of the alveolar process,

¹ To appear in the Journal of the American Dental Association, 1931.

apparently because its anterior end was deflected downward with the growth of the latter. In other cases he finds that the incisive foramen leads into a cylindrical canal from a few to ten millimeters in depth, the floor of which contains several foramina. Moreover, since every canal has two ends, he will wonder why we do not speak of inferior and superior incisive foramina.

Should the student turn to his Cunningham's Anatomy ('23), he will find it said that "The incisive canal passes downward through the bone (the maxilla) and divides into the foramina of Stensen and Scarpa, which open into the incisive foramen of the hard palate." He will read further that "The incisive foramen situated behind the incisor teeth, is a pit into which four small foramina or canals open. These openings are not always easily seen, especially if the incisive foramen is narrow and deep." Thoughtful students will naturally wonder how it is possible for a foramen to have three dimensions; how four foramina can open into one; or how a canal can divide into four foramina which open into a single incisive foramen! Perhaps he will have recourse to his dictionaries and read: "c. incisivus (BNA), incisive or incisor canal, anterior palatine canal; the lower, single, portion of the foramen incisivum" (Stedman, '30). Evidently an attempt is here made to reconcile the conflicting statements in the texts. Dorland ('25) tells him that the incisive foramen is "The aperture for the anterior palatine artery in the alveolar margin"; and that the incisive canal is "A passage in the upper maxilla from the incisive foramen to the nasal fossa"; and that "The incisor canal is the anterior palatine canal." These statements may well cause him to wonder whether the incisive and the incisor canals are one and the same thing or not. Further reference to his dictionaries will tell him that the incisor foramen is (Stedman): "The foramen of Stenson, a Y-shaped canal at the anterior part of the intermaxillary suture, having one opening below just behind the central incisor teeth, and two above, one on either side of the incisor crest; the upper arms contain the remains of Jacobson's organ."

If now he turns to Gray's Anatomy ('30), he will see a clear illustration of the palate from below, showing a large shallow oval depression in the anterior region, containing five approximately equal-sized foramina. Three of these are in the median sagittal plane and two somewhat lateral in position. The guide which carries the legend *foramen incisivum* points directly to the most anterior of the three median foramina.

If the student is not discouraged and has access to volume 4, part 1, of the "Elements of anatomy" of Quain ('15), he will learn that—

Close by the side of the incisive crest, on the upper surface of the palatine process, is seen a foramen, which is directed downwards to the mouth, but in the lower half of its extent becomes converted into a wider groove by deficiency of the median wall. Then when the two bones are placed in position, one orifice of considerable size is formed on the palatal aspect. The incisive foramen, which divides above into right and left branches leading to the corresponding nasal fossae, and called the incisive canals, or foramina of Stensen. . . . The incisive canals are placed between the two elements, the premaxilla and the maxilla proper. . . .

In Piersol ('30) it is stated that—

A little behind the incisors it (the maxilla) shows a groove in the lower part, which becomes a canal in the upper, and opens into the floor of the nasal fossa on either side. Thus there are two canals above and one below, like a Y placed transversely. These are the canals of Stenson, which transmit an artery connecting the vessels of the nose and mouth. Their common orifice is called the incisive foramen. Into this open two minute canals, the left anterior to the right, made by the junction of the bones. These are the canals of Scarpa and transmit the naso-palatine nerve.

In the fifth edition of Morris, undated, "The incisive foramen at the bottom of which are four foramina" is spoken of, which implies, as is common in our anatomies, that a foramen may have three dimensions, and the student may notice that figure 110 of the maxilla represents and designates only a groove which by inference will form a canal when joined to a similar groove on the right maxilla; and that, on page 89, the text speaks of "the foramina of Stenson, representing the

lower apertures of two passages by which the nose communicates with the mouth; they transmit some terminal branches of the descending palatine artery to the nasal fossae and lodge recesses of the nasal mucous membrane and remnants of Jacobson's organ." Spee ('96) states that two incisive canals join to form a common canal the lower aperture of which forms the incisive foramen; and Gould's dictionary ('28) will tell him that the incisor foramen is "The aperture of the incisor canal in the alveolar margin." Figure 172 of Toldt ('30) indicates an incisive canal, and figure 175, an incisive foramen, marked by the legend, "Anterior palatine fossa (into which open the incisor foramina, or foramina of Stenson)." The second edition of Toldt ('00) clearly represents an incisive canal and a foramen in conformity with the Basle terminology.

I might continue to quote from various contemporary sources available to the medical student, but I think any unprejudiced reader must recognize that there is considerable confusion in them regarding the terms here considered, and one cannot help but wonder when and how this confusion arose.

It is not possible to be certain regarding historical events, but it seems that Vesalius' anatomy is the oldest extant writing in which the incisive canals in man are described. I say extant, for one never can know what was lost, and in the days when human skulls were not an uncommon sight, many laymen other than gravediggers must have noticed the openings of these canals in skulls lying bleaching in the fields, supported on gate posts or on gibbets, or in those exhumed. Vesalius speaks of—

. . . . an opening near the back of the cutting teeth. There is made, by this opening, a connection and union of the membrane surrounding the palate with that which covers up the openings of the nostrils. A very small part of that membrane goes through it (the foramen), together with a small vein and artery.

Maar ('10) mistakenly calls Steno² the discoverer of the "ductus incisivi, s. nasopalatini, also called canales Stenoniani." and explains this discovery by saying that—

. . . . besides this infinite number of small glands he discovered in the sheep and in the dog a large, separate conglomerate nasal gland, and furthermore remembering how channels had been provided for carrying away the fluid of the eye, Steno looked for a passage into the mouth and found that the canales naso-palatini, sometimes called canales Stenoniani, might serve this end in about the same manner as the anterior and posterior apertures of the cavity of the nose. [!]

Although it is frequently so stated, I am unable to find that Steensen spoke of a duct in this region and, in writing regarding this communication between the nose and the mouth, he emphasized that it really is not a canal, but deserves to be called a foramen. "A naribus itaque in palatum qui patet, transitus brevis admodum est, nec canalis, sed foraminis nomine dignandus," and Maar says that Steensen himself listed it among his discoveries as "Meatus anterior e naribus in palatum."³

When considering these canals, Steno wrote:

Now that we have seen the vessels by which the nostrils are washed, we must investigate the ways by which this liquid is carried off elsewhere when it has performed its function. There are, of the kind which merit being examined here, two ways, one on either side. It is not the breathing passages of the nose, which are open for the

² Maar says that Steno's family name was Niels Steensen and that he usually used the Latin form Nicolaus Stenonis. He states further that the name also frequently is found in the Latin as Stenonius, in the French as Stenon, and in the Italian Stenone. Maar adds that the form Steno, which he uses, became established because it was thought that the genitive form was Stenonis. Le Double, on the contrary, says that: "Ce ne fut qu'après sa conversion au catholicisme qu'il prit le nom de Steno"—which may be correct, but is not necessarily of special significance.

³ I am not certain whether Steno discovered the parotid duct in the sheep and Sylvius that in man, before the former looked for an oral-nasal communication, but I believe he did. If so, his emphasis of the fact that the communication between the nose and mouth is not a canal or duct seems peculiar, especially so since he was looking for a passage which might convey secretion away from nasal glands. I surmise that he was influenced by what he had seen in sheep in this region, when he suggested that the incisive canal should be dignified with the term foramen instead of canal.

alternating movement of air, that are referred to here, since a healthy condition of life, when nothing is ever cleansed in this way, shows that these outer openings were not destined for this function; these, turning inward, hang out over the throat, and besides the fact that their size and use have made them familiar to all, so that it is superfluous to describe them here, their very position convinces that they are not adequate for this function. And so from the nostrils to the palate where it is open, there is a very short passage, not deserving the name of canal, but of foramen. If you seek the place, you will find it in the end of the nose, where the cartilage, rising above the edge of the third jawbone, keeps the flowing liquid from running out. Within this barrier in man you will find this round opening, very near to the side of the vomer, and although it is large enough at its upper edge, nevertheless it is so contracted that it permits the passage of not even a hair into the palate. If you look at the roof of the palate, in man, and also in dogs, you will see a swelling rising at the roots of the front teeth, on each side of which, if you press the membrane ever so little, a drop will come forth and show a connection of the mouth with the nose. . . . But in various skulls the formation of this passage is not the same; in man, the openings are seen to be small and divided in the cavity of the nose by the vomer in the middle, and if you invert the jawbone, you find that the passages have united into one, below the teeth. In animals however, they remain divided, and are not round but extended in oblong openings.⁴

Sömmering (1791), in enumerating the canals in the upper jaw, spoke of the second canal as—

Den Kanal hinter den Schneidezähnen zum Durchgange eines Nerven vom Zweiten Aste des Fünften Paares und einiger Blutgefäße, die eine Gemeinschaft zwischen den Gefäßen der Nasenhöhle und des Gaumens unterhalten. Bald ist dieser Kanal sehr weit, bald sehr enge; bald bilden beide Oberkiefer Zusammen einen nur oben oder anfangs, bald hingegen einen durchaus gemeisschaftlichen Kanal; bald bildet jeder vorne einen eignen und überdies entweder hinten oder vorne, oder zugleich hinten und vorne einen mittleren gemeisschaftlichen Kanal, so dass man an dieser Stelle von unten her im zusammenhängenden Schedel vier Löcher sieht. Bisweilen ist selbst, wenn noch alles mit Haut überzogen ist, hier ein offener Kanal.

⁴I am indebted to Miss Helen Yeomans for this translation and that from Vesalius.

Monro (1801) wrote:

The second (aperture) is the foramen incisivum, just behind the fore teeth; which, at its under part, is one irregular hole common to both the maxillary bones when they are joined; but as it ascends, soon divides into two, three, or sometimes more holes; some of which open into each nostril. Through them small arteries and veins, and a twig of the second branch of the fifth pair of nerves, pass, and make a communication between, or join the lining coat of the nose and mouth. In some subjects, Steno's duct may be traced some length on the side of these passages next to the nose, and small orifices may be observed opening into the mouth.

Henle (1817) mentioned a "Foramen incisivum" and the "Apertura sup. Can. incisivi." and Bell (1827) stated that—

A hole in the palate plate, which belongs equally to each of the maxillary bones, may be counted the second foramen; for it is between the two bones in the lower part, or beginning of the palate suture behind the two first cutting teeth. This hole is named foramen incisivum, as opening just behind the incisive or cutting teeth; or it is named anterior palatine hole, to distinguish it from one in back of the palate. This hole is large enough to receive the point of a quill; it is single toward the mouth, but toward the nose it has two large openings, one opening distinctly into each nostril.

Enumerating the three foramina in the "Ossa maxillaria superiora," Wistar (1830) spoke of the second foramen as—

The foramen incisivum or anterior palatine hole, which passes through the palatine process from the nose to the mouth. In the nose there are generally two foramina, which unite to form but one in the mouth, immediately behind the incisor teeth. This foramen is enclosed by the soft parts during life, and transmits a branch of the sphenopalatine nerve from each side, which runs on the septum narium, and joining at the lower part of the canal with its fellow, they unite, and according to Cloquet, form a ganglion.

Arnold ('45) wrote as follows:

Zur Seite des vorderen und höchsten Theils des Nasenkammes findet sich an der Nasenfläche ein Loch, welches in eine Furche am inneren Rand des Gaumenfortsatzes übergeht; durch die Vereinigung beider Oberkieferbeine entsteht ein Kanal, canalis incisivus, der mit zwei Oeffnungen in der Nasenhöhle beginnt, dann einfach wird, schräg nach vorn und unten läuft und mit einem Loch, dem *vorderen Gaumenloch*, foramen palatinum anterius s. incisivum, am

Gaumen in der Mitte und dicht hinter den Fächern für die inneren Schneidezähne mündet. Durch diesen Kanal gehen die Enden des Nasescheidewandnerven, sowie die vorderen Gaumengefäße.

Wilson ('53) wrote:

At the anterior extremity of the hard palate, and directly behind the front incisor teeth, is the anterior palatine or incisive foramen, the termination of the anterior palatine canal, which contains the naso-palatine ganglion, and transmits the anterior palatine nerves.

Quain ('82), volume 1, ninth edition revised by Thane, designates the common canal, or what is commonly called the incisive foramen or anterior palatine fossa, as the anterior palatine, and Stenson's canal as the incisor foramen (or canals); but the tenth edition, 1896, contains the following description:

Close by the side of the incisor crest (of the maxilla) on the upper surface of the palate plate is seen a foramen which is directed downwards to the mouth, but in the lower half becomes converted into a wider groove by deficiency of the inner wall. Thus, when the two bones are placed in apposition, one orifice of considerable size is formed on the palatal aspect, which divides above into right and left branches leading to the corresponding nasal fossae; the lower aperture is the *anterior palatine fossa* (or *canal*), the lateral branches are the *incisor foramina* (or *canals*) or *foramina of Stensen*. Farther, in the middle line are two other smaller foramina opening into the anterior palatine fossa, one before, the other behind; these are the *foramina of Scarpa*.

Nicolas, in Poirier et Charpy, 1911, volume 1, gives the typical arrangement of the four canals and logically distinguishes them as anterior, posterior, and lateral. He describes them as follows:

Entre les bords internes des deux apophyses palatines du maxillaire est ménagé un canal, le *canal palatin antérieur* ou *incisif* (*foramen incisivus BNA*), qui s'ouvre en haut dans les fosses nasales, et en bas dans la cavité buccale, au niveau de la fosse incisive. Si l'on regarde au fond de celle-ci avec attention, on constate que, dans plus de la moitié des cas, ainsi que l'avait bien vu Scarpa (*Liber secundus anatomicarum annotationum*), elle est percée non pas d'un trou unique, mais de quatre orifices placés en croix.

Since Vesalius wrote of a foramen close to the back of the incisor teeth of the upper jaw, the retention of the term

foramen incisivum was a just tribute to him and I have no desire to deny him the least credit or honor. His fame is secure and the retention of the term is possible, though it is not the most preferable, because it would be well to adopt a consistent terminology even though the rest of the Basle terminology be not consistent in all other respects.

Since the Basle terminology does not recognize the depressions on the anterior; ventral; surface of the jaws or list them as incisor fossae, this designation is available for the fossa so commonly present in the anterior palatine region, into which the anterior palatine canals open. However, since the Basle terminology retains the term *juga alveolaria* and since the term *incisor fossa* is such an old one, and also because the term *canine fossa* is retained in that terminology, it probably would be very difficult to displace the term *incisor fossa* used so long to designate various depressions on the anterior surface of the jaws. The difficulty could be met, however, by adding a distinguishing adjective to the term *incisor fossa*, as we do in the case of the *biceps* and *triceps* and so forth. We could speak of *maxillary*, *mandibular*, and *palatine incisive fossae*, for example, and we could also speak of *median* (*Scarpa*) and *lateral* (*Stensen*) *incisive canals* and of the *superior* and *inferior foramina* of these.

Whenever the major anterior palatine, or incisive canals, do not open upon a plane surface of the palate as they do in about half the cases, as indicated by an unpublished survey made by Howard in this laboratory, we could then speak of them as opening into the palatine incisive fossa or into a common palatine canal. However, since the terms *major* and *minor* have been retained in the Basle terminology in connection with the posterior palatine canals, there would seem to be no good reason for not using them also in connection with the anterior palatine canals. The terms *anterior palatine foramen*, *fossa*, and *canals* can be used to distinguish these structures in the anterior palatine region, as we use them for structures in that region of the palate. This is consistent and most preferable and will avoid confusion.

We would then speak of the canals of Scarpa as the minor anterior palatine and those of Stensen as the major anterior palatine canals, and of the single canal or fossa common to them as the anterior palatine canal or fossa. This would enable us to abandon the proper names Scarpa and Stensen and the confusing and inadequate names incisive foramen and canal and would make the terminology in the anterior region of the palate correspond to that in the posterior region. This suggestion also makes it unnecessary to use the term incisive fossa for various things in this region or the term incisive foramen with ambiguity.

Our texts and atlases now use the expression incisor fossa for three different depressions upon each maxilla and for two on the mandible, and that would seem quite a sufficient number of things to be designated by one term. Moreover, these depressions really are much more in the nature of fossae than most of those in the anterior region of the palate, into which the anterior palatine canals often empty. The suggested nomenclature would not meet all difficulties, but those that remain would be of a purely minor nature and be due to the fact that some of the canals may join or be absent. The designation major anterior palatine canals and canal would not enable one to distinguish between the common canal formed by the two major palatine canals, for example, and the common canal into which the united or single, major palatine canals and the minor palatine canals sometimes empty. The canal common to the major palatine canals, and sometimes to all of them, could be spoken of as a fossa, but that violates the underlying idea implied in the word fossa. However, since we must now use the term foramen for both of these things and for the fossa as well, the difficulties that would remain upon the adoption of the suggested terminology are purely minor. Moreover, I am tempted to add that the quotations given in this discussion show that most text-book writers do not know the anterior palatine region well enough to find the suggested terminology inadequate!

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THE DEVELOPMENT OF THE SUPRARENAL GLAND IN THE ALBINO RAT, WITH A CONSIDERATION OF ITS POSSIBLE RELATION TO THE ORIGIN OF FOETAL MOVEMENTS

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ELEVEN FIGURES

In this preliminary publication a study of the development of the suprarenal gland is presented, from the time that the gland first appears in embryos of higher vertebrates to the end of foetal life. Special attention has been given to the migration of sympatho-chromaffin cells and the development of associated nerve fibers, since it was early noted by the author that these processes immediately precede the appearance of foetal movements and may, therefore, be causally related to the latter. In the present article attention will be focused on the coincidence in development of the suprarenal gland and foetal movements.

MATERIAL

Through the courtesy of the staff of The Wistar Institute in Philadelphia, an extensive collection of serially sectioned rat embryos was made available to the author. The material also included some rats that Angulo ('28) had studied and other series especially prepared by the institute for this investigation.

The writer wishes here to express his appreciation and gratitude to the staff of The Wistar Institute of Anatomy and Biology and especially to Dr. G. E. Coghill, who first suggested the problem and whose candid, kind, and mature criticism has made this work possible.

OBSERVATIONS

The suprarenal glands in the albino rat appear first in the thirteen-day-old foetus. In the twelve-day-old foetus no thickening of the epithelium in the dorsal wall of the coelomic cavity can be seen (fig. 1). A slight thickening of the coelomic epithelium medial to the cephalic portion of the mesonephric fold marks the anlage of the suprarenal cortex. This process is due to a proliferation of coelomic epithelium in a fold which is continuous with the pleuroperitoneal fold cephalically and with the genital fold caudally. This is described in man by both Zuckerkandl ('12) and Wieman ('20). The

ABBREVIATIONS FOR ALL FIGURES

<i>Ao.</i> , aorta	<i>P.</i> , pleuroperitoneal cushion
<i>Ad.An.</i> , adrenal anlage	<i>P.C.</i> , pleuroperitoneal cavity
<i>Ad.C.</i> , adrenal cortex	<i>P.Ep.</i> , peritoneal epithelium
<i>B.C.</i> , blood cells	<i>S.</i> , stomach
<i>C.C.</i> , cortical cells	<i>Sp.</i> , spleen
<i>C.V.</i> , central vein	<i>Sup.</i> , suprarenal
<i>G.C.</i> , ganglion cells	<i>Sup.L.</i> , suprarenal ligament
<i>L.</i> , liver	<i>Sy.C.</i> , sympatho-chromaffin cells (medulla)
<i>M.</i> , mesentery	<i>Sy.C.R.</i> , sympatho-chromaffin 'rosette'
<i>Ms.</i> , mesonephros	<i>Sy.G.</i> , sympathetic ganglion
<i>M.T.</i> , mesonephric tubules	<i>V.</i> , vertebra
<i>N.F.</i> , nerve fibers	
<i>N.Spl.</i> , splanchnic nerve	

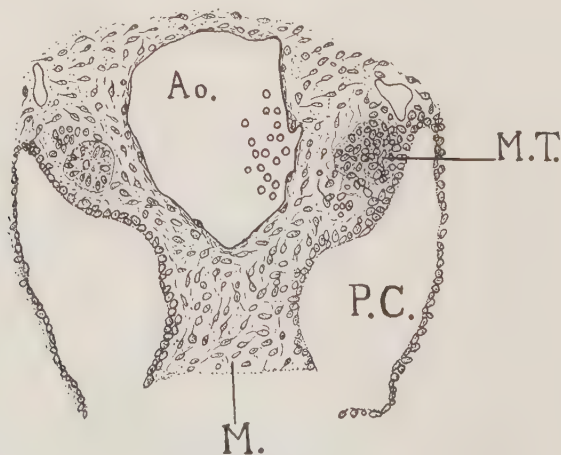


Fig. 1 Transverse section through a twelve-day-old rat foetus. $\times 330$.

epithelial cells in this region become definitely larger, their nuclei vesicular, and they appear to migrate into the mesenchyme dorsally (fig. 2). This process in the rat is much like that described in the bird by Hays ('14), in the pig by Whitehead ('03) and Weyman ('22), and in man by Zuckerkandl ('12) and Fischel ('29). Such a complex development of the cortex as described in man by Keene and Hewer ('27) was not observed in the rat.

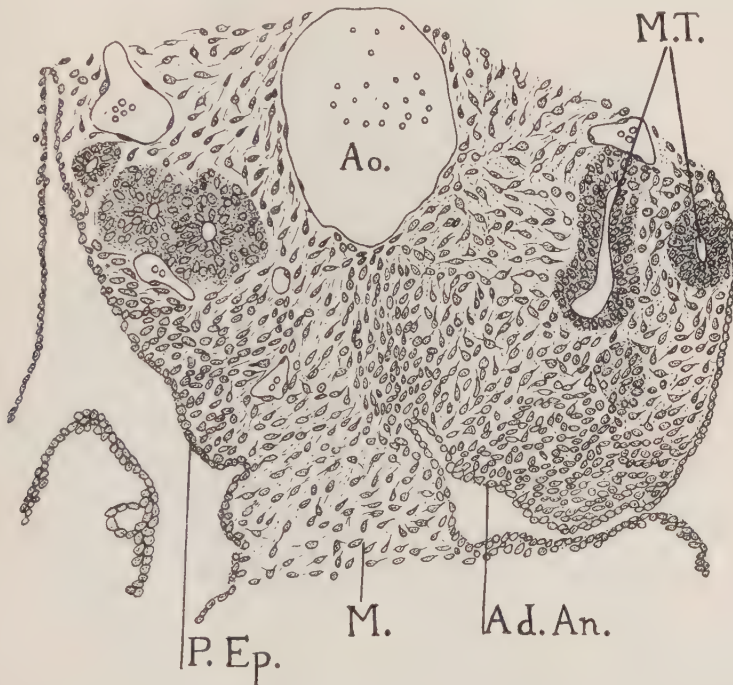


Fig. 2 Transverse section through a thirteen-day-old rat fetus. $\times 330$.

In the fourteen-day and fifteen-day fetuses the thickening of the epithelium and its dorsal migration were more apparent (figs. 3 and 4). In these stages one could also see the sympathetic trunk and its ganglia, the aorta, mesonephric tubules, and stomach in their relation to the suprarenal anlage. Growth at this time is rapid, as the figures indicate. During the fifteenth to the sixteenth days the cortex forms a

marked oval mass which protrudes into the dorsal coelomic cavity (figs. 4 and 6). This corresponds to the 25-mm. stage of man described by Keene and Hewer ('27). At this stage of development of the cortex, there is a 'breaking up' of the cortex by small blood vessels, which, according to Wieman ('20), probably later form the central veins (fig. 4).

In the sixteen-day fetuses, shortly before motility was observed (Swenson, '27), the cortex is very spongy, due to

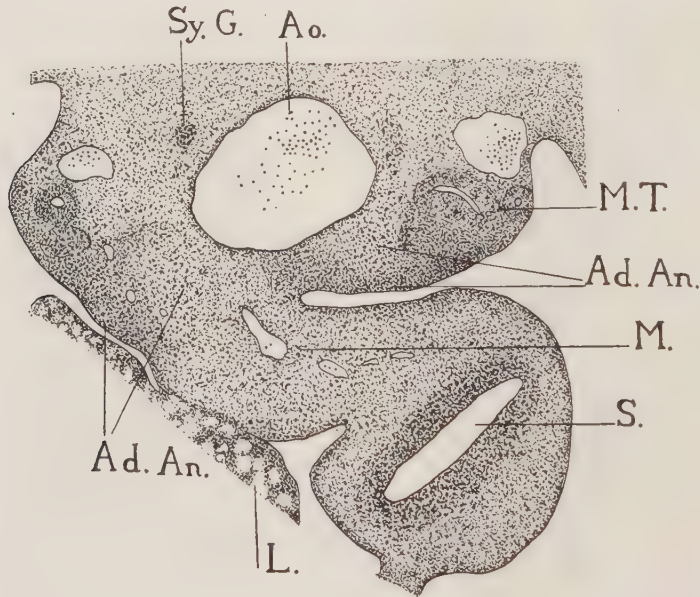


Fig. 3 Transverse section through a fourteen-day-old rat fetus. $\times 200$.

the numerous vascular spaces which later are invaded by sympatho-chromaffin cells (fig. 6). About the sixteenth day, small darkly staining cells migrate ventrally from the sympathetic ganglia toward the cortex. Soulié ('03) describes the beginning of penetration of the cortex by the sympatho-chromaffin cells in a 15-mm. rat embryo and their reunion into a central nucleus in a 25-mm. embryo.

Just before movements were first observed, masses of small darkly staining cells can be seen on the medial surface of the

cortex. Sometimes one may find even a definite mass of these cells in this position (fig. 8). At the time of first motility small clumps of the migrating cells are found lying in the vascular spaces of the medial part of the cortex. In the defi-

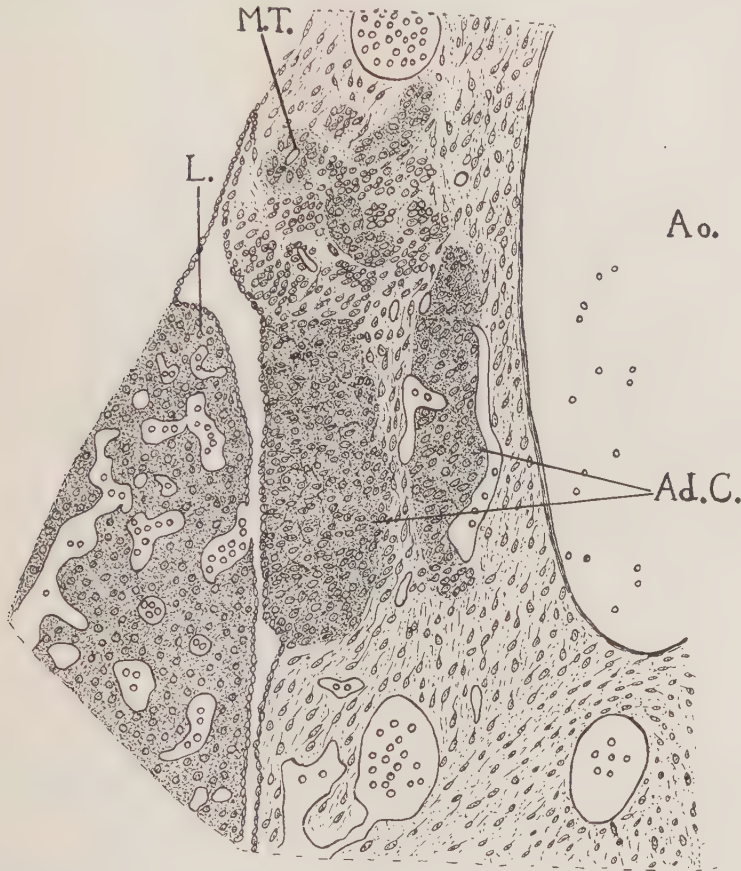


Fig. 4 A nearly longitudinal section through a fifteen-day-old rat foetus.
 X 330.

nitely motile foetuses (sixteen days) I often observed a marked bundle of nerve fibers and ganglionic cells along their course (figs. 6 and 6a).

Fischel ('29) says that the suprarenal gland in man remains connected with the sympathetic chain by the processes of

these migrating cells which eventually form the medulla. The processes on some of the large ganglion cells (*G.C.*, fig. 6a), observed in only a few cases, appear to corroborate his observations. Apparently there are two types of sympatho-chromaffin cells that migrate into the cortex besides the comparatively few large ganglionic cells. The small darkly

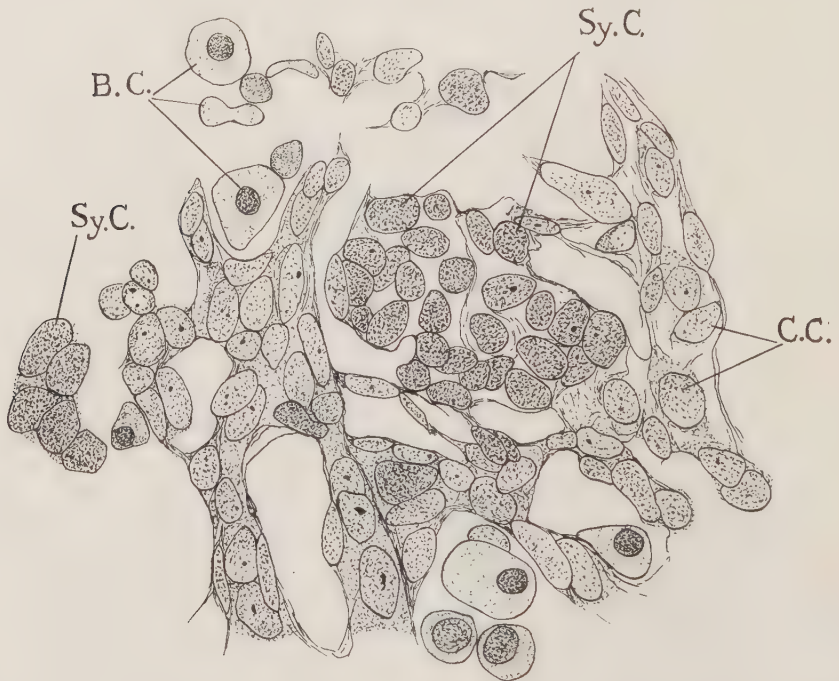


Fig. 5 A section through the suprarenal cortex of a non-motile sixteen-day-old rat foetus, showing a mass of sympatho-chromaffin cells in a vascular space. $\times 2000$.

staining cells are the most numerous, especially in the early stages of cell migration. Then there are larger and paler cells with somewhat vesicular nuclei and cytoplasmic processes (fig. 6a). For a more detailed description, the reader is referred to the article by Keene and Hewer ('27).

In all the rat series examined (about twenty-five series) only a few large ganglionic cells were found in the medulla

of various ages up to birth (fig. 10). On the other hand, I have often observed 'rosettes' of darkly staining cells in the suprarenal gland of the actively motile rat foetuses. These have been described in man by Keene and Hewer ('27). Figure 9 shows such a 'rosette' with a bundle of nerve fibers connected to it.

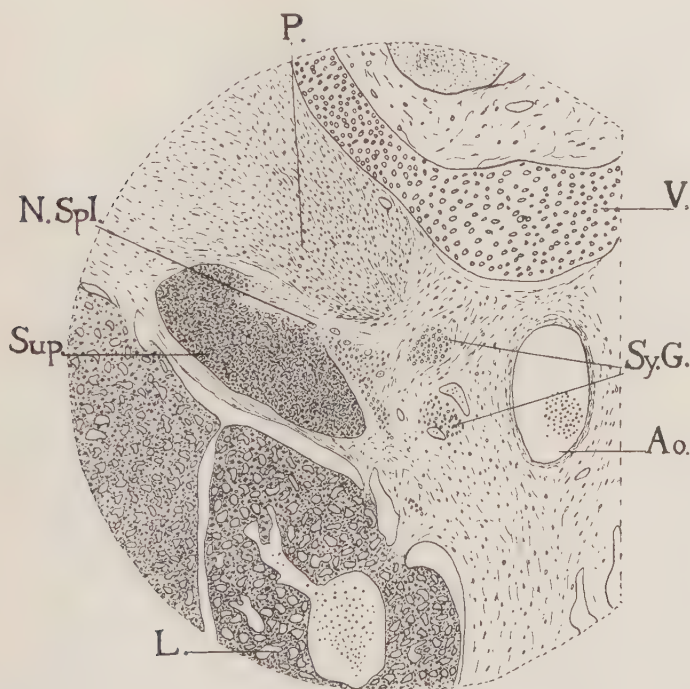


Fig. 6 Transverse section through a motile sixteen-day-old rat foetus. $\times 100$.

This cell migration, which began during the sixteenth day, continues during the following days, apparently till birth (figs. 6, 6a, 7, and 8). In all the foetuses of seventeen, eighteen, and nineteen days definite nerve connections to the gland could be seen (figs. 6, 7, and 8). Mitsukuri ('82) shows, in figure 7, a 23-mm. rat embryo with nerve fibers passing into the suprarenal mass. Soulié ('03) gives a short description, but does not show any figures for the rat.

In the rat at birth a very definite nerve (*N.Spl.*, fig. 11) connects the suprarenal gland with the sympathetic chain. The medullary cells are found around and in the vascular spaces, closely crowded against the cortical cells (fig. 11). Fischel ('29) describes such a relation in the suprarenal in man.

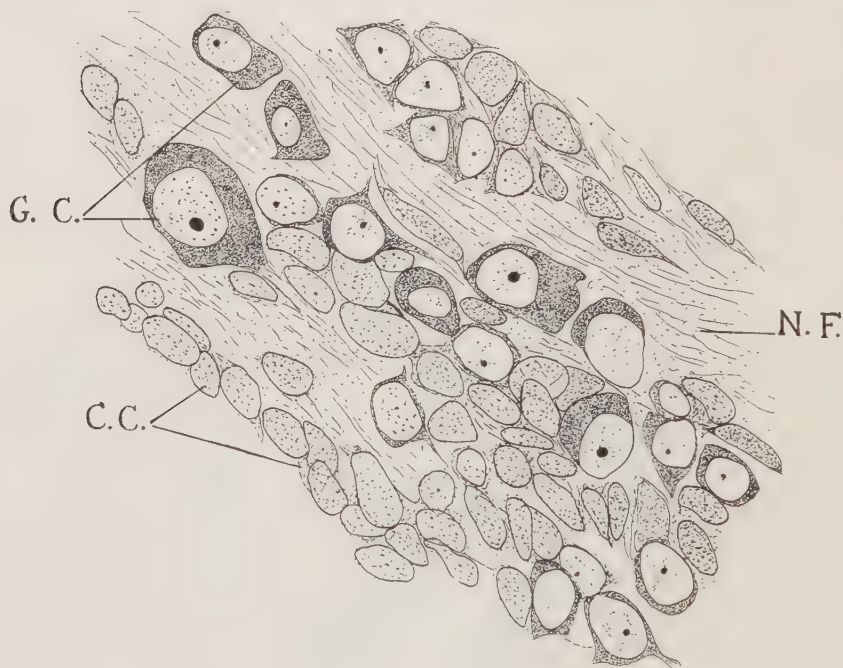


Fig. 6a A section showing a higher magnification of the migrating cells of section shown in figure 6. $\times 2000$.

DISCUSSION

A survey has been made of the literature dealing with the development of the suprarenal gland, the migration of sympatho-chromaffin cells, the first chromaffin reaction, and the origin of foetal movements. Numerous writers, notably Soulié ('03) and Lutz and Case ('25), state that undoubtedly the adrenal hormone is present and capable of functioning at a very early age in the mammalian foetus. They also call attention to Fenger's ('12) observations which suggest that

the suprarenal glands have a relatively more important rôle in the embryo than in the adult. From the phylogenetic point of view Gaskell ('19) points out the fact that in the lowest vertebrates the function of the sympathetic nervous system is largely taken over by a chromaffin system. Such facts give credence to the view that in the embryos of higher vertebrates

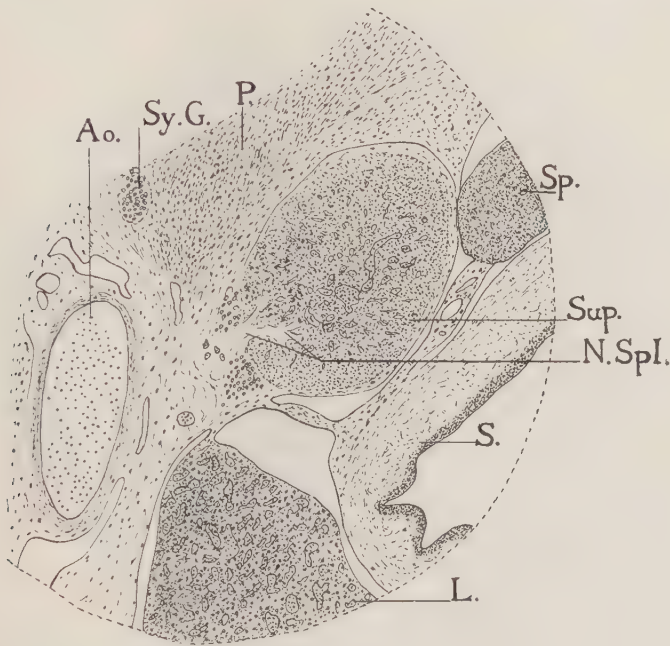


Fig. 7 Transverse section of a seventeen-day-and-five-hour-old rat foetus (motile). $\times 100$.

the suprarenal medulla may function before the sympathetic nervous system.

Probably the most complete description of all phases of investigation is available for the chick. Preyer ('85) was the first investigator to study the embryonic movements of the chick extensively. The first movements were noticed in a five-day-old embryo and consisted in a bending of the back from side to side. Later, Clark and Clark ('14), with more refined methods, further studied the development of the

embryonic movements in the chick. Their findings corroborate Preyer's work. They describe the early stages as consisting of passive movements of the head, tail, and extremities. They further observed that during the sixth and seventh day the bending of the body becomes more pronounced, the tail contracts independently, the head nods, and the

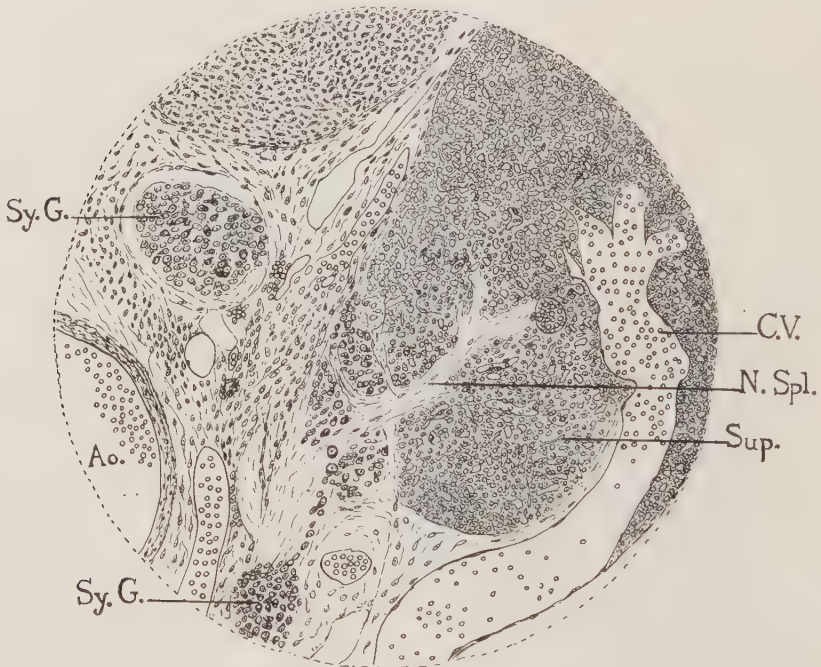


Fig. 8 Transverse section of an eighteen-day-and-six-hour-old rat foetus (motile). $\times 330$.

paddle-like extremities are moved inward and outward. From the eighth to eleventh day, the movements continue to increase in strength and variety.

In the same year, Hays ('14) described the development of the adrenal glands in birds, using the chick. He states that the first evidence of any connection between the anlagen of the prevertebral sympathetic plexuses and chromaffin substance is seen after 130 hours of incubation (5.4 days). After

168 hours (seven days) of incubation, the cells which are to form the medulla of the gland are beginning to show some differentiation. During the next twenty-four hours, or the eighth day, the chromaffin cells within the glands have increased greatly in number, and most of the sympathetic cells have disappeared from the surrounding mesenchyme. The arrangement of the chromaffin cells undergoes a marked change during the ninth day of incubation. At the same time

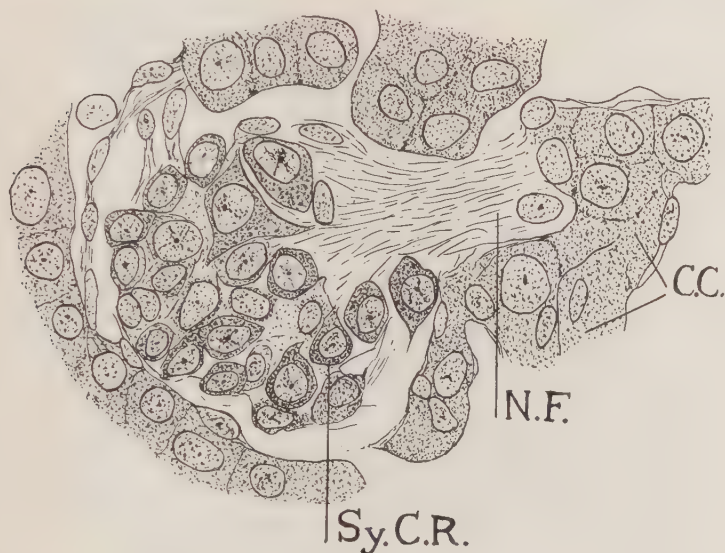


Fig. 9 A section showing a sympatho-chromaffin 'rosette' in an eighteen-day-and-six-hour-old rat foetus (motile). $\times 1500$.

the chromaffin cells are found in small groups arranged around the venous blood vessels. Furthermore, during the period in which there is the greatest influx of cells from the anlagen of the prevertebral sympathetic plexuses there is also the greatest activity in the development of the vascular systems. Later, Lutz and Case ('25) and Okuda ('28) investigated the appearance of adrenalin in the chick by biological and chromic reaction tests. Using the enucleated frog's eye, Lutz and Case ('25) obtained the first positive adrenalin reaction in seven-day-old chick embryos. Okuda ('28) used

Cannon's intestinal method and the chromic-acid reaction. He says:

. . . in the chick embryo the suprarenal adrenalin appears on about the eighth day of incubation, and chromic reaction of the gland cells sets in at nearly the same time. Their intimate relation which Ogata ('16) pointed out, is thus confirmed. The adrenalin content of the glands increases gradually with the progress of development in an incubator, but a more or less marked increment is found on the 13th to 21st days.

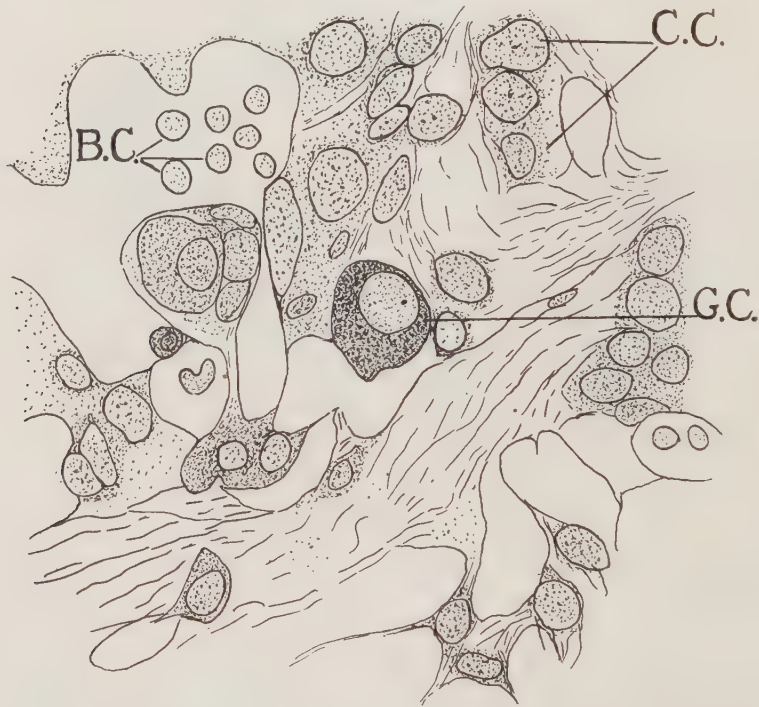


Fig. 10 A section showing a ganglion cell in the suprarenal gland of an eighteen-day-and-four-hour-old rat foetus (motile). $\times 1500$.

In the chick we see that the positive tests for adrenalin appeared a few days after the migration of the sympathochromaffin cells. On the basis of Miller's ('26) and Weyman's ('22) observations, possibly the actual beginning of the formation of adrenalin in the chick takes place somewhat earlier

(fifth to sixth day) than has been described at the present. From other investigations it appears that there is a production of adrenalin even during the later part of the sympathochromaffin cell migration.

Concerning the development of the suprarenal medulla and the appearance of adrenalin in the mouse, Miller ('26) gives

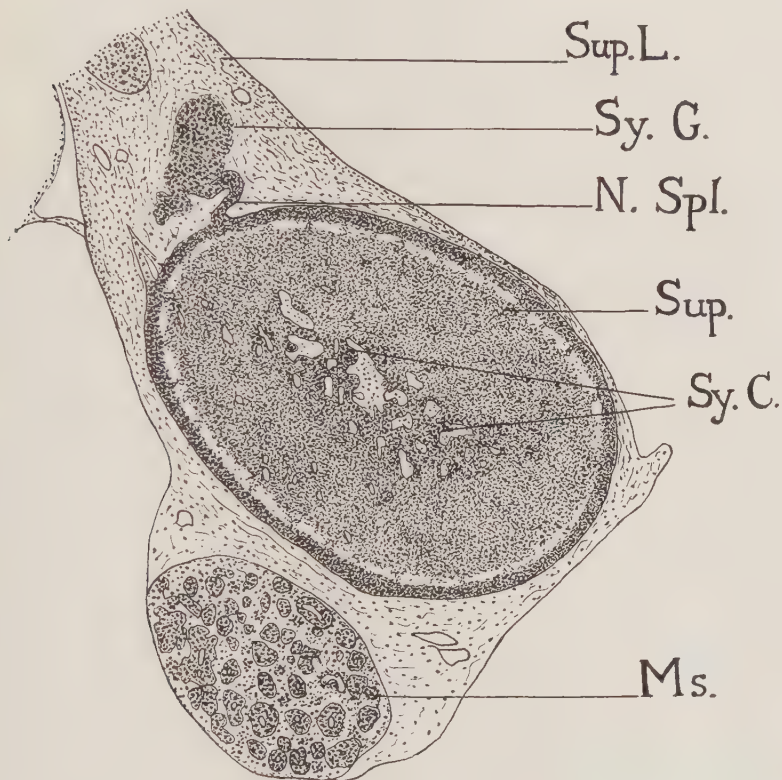


Fig. 11 Transverse section through a rat at birth. $\times 70$.

a full account. According to her observations, the first appearance of the chromaffin reaction takes place between the latter part of fourteenth day and the first half of the fifteenth day. "This," she states, "is at a time when the future medullary cells are just beginning their penetration between cortical cells, leaving the sympathetic ganglion anlage, or

sympatho-chromaffin mass, which is closely applied to the suprarenal cortex at this time." Her observations are based on both the chromic reaction and Cannon's intestinal method. She also observed that the seventeen-day fetuses were highly motile.¹ Inaba ('91) gives probably the earliest account of the development of the suprarenal in the mouse. He gives figures showing the final nerve connections of the gland.

Some observations have also been made on the rabbit. According to Kölliker, the suprarenal bodies in the rabbit appear first, on the twelfth or thirteenth day of gestation, as masses of somewhat large round cells on each side of and ventral to the aorta. On the fourteenth day the suprarenals are already well marked (Mitsukuri, '82). They consist of a mass of cells with large nuclei, divided into indefinite cords by blood capillaries which are already somewhat numerous. Mitsukuri ('82) states that at this stage the connection of the gland with the sympathetic chain, by means of a mass of cells, is not so obvious as later on. But in the suprarenals of the sixteen-day-old embryos great changes were observable. Mitsukuri says that the medullary part (*m.*, fig. 5) surrounded for the most part by the cortical substance (*c*) can now be clearly distinguished. In the same figure he shows a branch (*a.*, fig. 5) of the nervous mass (*n*) passing into the suprarenal and uniting with the medullary substance (*m*). Minot ('97) describes a similar development of the suprarenal in the rabbit. Kohn ('03) noted the presence of the chromic reaction as early as the period of beginning migration of the future medullary cells into the suprarenal cortex. In the fourteen-day fetuses he was unable to detect any chromaffin cells. In fifteen-day rabbit fetuses he first observed the chromaffin cells lying medially to the cortex; and in the sixteen-day fetuses the reaction was very definite and the sympatho-chromaffin cells are penetrating the cortex. This penetration, according to Kohn ('03), is much more rapid in the rabbit than in man. Soulié ('03) likewise says that about the end of the fourteenth day one is able to see a mass of

¹Information on motility was given to the author by personal communication.

sympathetic ganglion cells in contact with the cortex. About the fifteenth day the cortex receives a definite fasciculus of fibers, but is not penetrated by the future chromaffin cells.

The first stage of motility—in the form of ventrolateral flexion of the head and upper trunk (as described by Swenson ('27) in the rat and by Windle ('30) in the cat)—has been first observed by the writer in fifteen-day and seventeen- to twenty-hour rabbit fetuses.²

The observations on the cat seem to suggest a similar relation. Zuckerkandl ('12), speaking of the development of the suprarenal in man and in the cat, says, "thus, for example, in an 11 mm. cat embryo the suprarenal ridge does not project as much as in man." He further compares an 11-mm. human embryo as being similar to a 10-mm. cat embryo in suprarenal development. Kohn ('03) first differentiated the sympatho-chromaffin cells in the ganglionated chain in a 12-mm. S.S.L. cat embryo. Soulié ('03), in his description of the development of the suprarenal of the cat, says:

. . . . cet mas, d'abord homogène, paraît composé de nodules secondaires sur les embryons de 14 mill., et prend un aspect nettement réticulé sur les embryons de 16 mill. C'est entre les stades 16 et de 18 mill. que commence la pénétration des éléments parasymphatiques, cette pénétration est à peu près achevée sur les embryons de 4 cent.

Recently, Windle ('30) has observed the first foetal movements in cat fetuses 17.5 and 18.5 mm. in length.

In man the status of our information is in an unsettled condition. Soulié ('03), Keene and Hewer ('27), and Fischel ('29) state that the cortex in man appears in a 5-to-6-mm. embryo. Other investigators give slightly different ages. Soulié ('03) says that the cortex develops rapidly, and in a 19-mm. embryo. According to Fischel ('29), the migration of sympatho-chromaffin cells into the cortex takes place in 14- to 20-mm. embryos. Keene and Hewer ('27) describe and figure the beginning of such a migration in a 12-mm. embryo, and show that it becomes prominent in twelve- to twenty-two-week embryos and ceases at full term. In the 25-mm. stage,

² The data on these observations have not been published.

according to their observations, the suprarenal gland is defined and consists of true cortex, foetal cortex, and immigrating sympatho-chromaffin bundles. Jordan and Kindred ('30) also state that the migration begins at about the 19-mm. stage and continues until after birth. Gradually the primordial chromaffin cells surround the central vein, which appears at about the 23-mm. stage, and ultimately differentiate into characteristic chromaffin cells.

Various early investigators have failed to find any adrenalin in the suprarenal gland of human foetuses. On the other hand, recent works show that the adrenalin begins in relatively young foetuses. Hammar ('25) says that the chromaffin cells appeared first in an embryo of 22.2-mm. length, and that at that time the paraganglionic cords began to be distinguishable. According to Keene and Hewer ('27), adrenalin appears at twelve weeks, and the chromaffin reaction appears at twenty-two weeks. Fenger ('12) furthermore says that in every case one finds adrenalin stronger relatively in the embryo than in the adult.

The first motility in the human foetuses has been observed as follows: Motility in the arm at 2 cm. C.R. (Yanase, '07); spontaneous motility in the arms and legs at 2.2 cm. C.R. (Strassmann, '00-'03); and oral reflex, with associated leg movement, at 3.5 cm. C.R. (Minkowski, '28). After this age, other investigators (Bolaffio and Artom, '23-'24; Erbkam, '37; and Krabbe, '12) have noted various types of early foetal movements. For a more detailed discussion of these movements the reader is referred to Coghill's paper ('29). Due to lack of material and uniform standard of age and lengths, when dealing with human material, definite conclusions cannot be readily formulated.

The latest investigations in the vertebrates studied point to a definite relation between the migration of sympatho-chromaffin cells, the chromic reaction, and the appearance of adrenalin. Likewise, foetal movements, in the forms studied, apparently begin at approximately the same stage of development. This striking parallelism has suggested a possible

relationship of cause and effect which is now being investigated in a number of different mammals.

Tabulated summary of the data and discussion

	THE MIGRATION OF SYMPATHO-CHROMAFFIN CELLS	THE CHROMIC REACTION	THE ADRENALIN REACTION	EARLY MOTILITY
Chick	130 hours; 5.4 days	8th day	8th day	5th day
Mouse	14th-15th day	14th-15th day	14th-15th day	Very motile at 17 days 16th day
Rat	15th-16th day	?	?	16th day
Rabbit	15th-16th day	?	?	15th-16th day
Cat	16-18 mm.	?	?	17.5 and 18.5 mm.
Man	14-20 mm.	22 weeks	12 weeks	2-3.5 cm.

CONCLUSION

The suprarenal gland in the albino rat appears first in the thirteen-day-old foetus. A slight thickening of the coelomic epithelium medial to the cephalic portion of the mesonephros marks the anlage of the suprarenal cortex. During the fifteenth to the sixteenth day the cortex forms an oval mass which protrudes somewhat into the dorsal coelomic cavity. About the sixteenth day, just before foetal movements begin, cells migrate from sympathetic ganglia toward the cortex. At the time (sixteen days or more) when foetal movements have first been observed, a definite mass of sympatho-chromaffin cells is located among the medial cell cords of each cortex. This cell migration continues during the seventeenth and eighteenth days and possibly till birth. In seventeen-, eighteen-, and nineteen-day foetuses nerve fibers which connect the gland with the sympathetic chain were observed with large ganglionic cells along their course. From the literature on the different phases of the development of the suprarenal gland and appearance of foetal movements in the chick, mouse, rabbit, cat, and man, the author concludes that there is in these animals a coincidence in development of gland and foetal movements similar to that which obtains in rat embryos. These facts have suggested that the beginning of foetal movements may, perhaps, be facilitated by hormones of the embryonic gland in some such way as adrenalin is

known to affect the threshold of skeletal muscle in adults—a hypothesis that is now being subjected to experimental methods.

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A PERSISTENT RIGHT OVIDUCT IN THE DOMESTIC FOWL¹

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TWO FIGURES

During recent experimental observations relative to the etiology and transmission of certain diseases in domestic fowls, about 450 chickens of various breeds and ages came to necropsy. In this rather extensive series two chickens appeared which presented certain anatomic variations of the genital tract which were of unusual interest. Normally the left ovary and the left oviduct persist and become functional in the mature hen, but in the two cases forming the basis of this report both right and left oviducts had persisted, and in one of the two the right duct was apparently functional.

The first case which was encountered occurred in an adult barred Plymouth Rock hen dying from myelogenous leukemia. There was no indication of a right ovary macroscopically, but the left ovary was normal, relatively quiescent, without mature follicles, and was situated in the usual anatomic position. The oviducts were unusual, however, in that the right and the left had both attained mature proportions (fig. 1). When relieved of its convolutions, the left duct was approximately 18 cm. in length, the average for an adult non-producing hen. The persisting right duct was as well developed as the left and comparable in length and diameter to a normal oviduct. Under normal conditions the free fimbriated extremity with its ostium is suspended by the broad ligament, whereas in the case of this persistent right duct all fimbriation

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was absent and the tube ended blindly, fixed by a small band of fibrous tissue to the dorsal peritoneum, exactly opposite the level of the left ovary. Except for the anterior 3 cm., the right duct was patent throughout and opened exactly opposite the opening of the left duct.

The second case occurred in a White Leghorn hen aged two years. In this bird the ovary was enlarged and diseased,

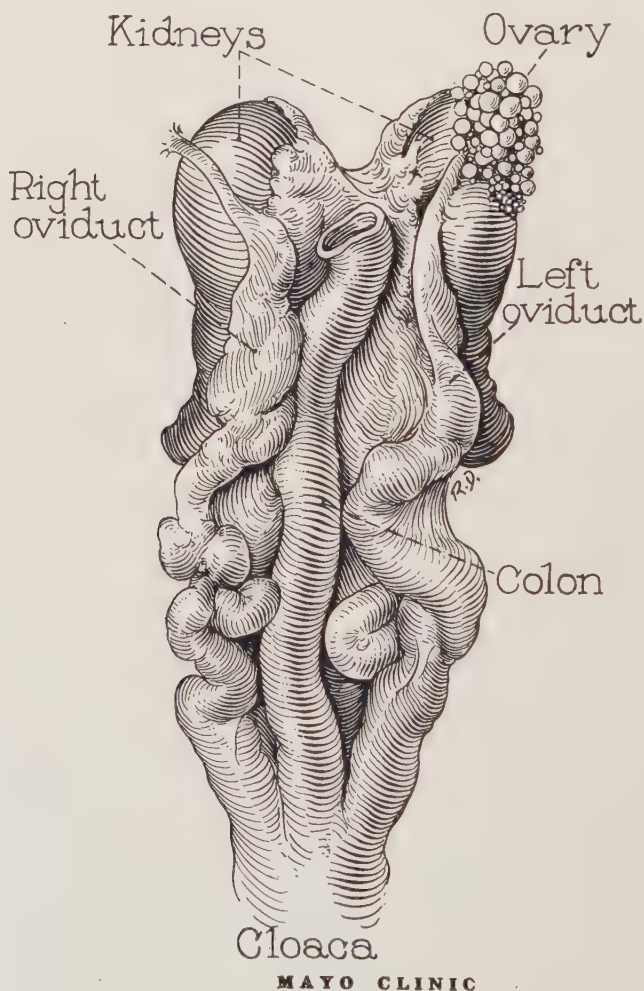


Fig. 1 Case 1. Illustrating equal development of right and left oviducts.

but was situated in the normal position on the left side. It contained several mature ova, showing that the ovary was functioning. Macroscopically, there was no evidence of a persistent right ovary. The left duct was partially distended and average in size for a producing hen and was situated in the normal position to the left of the median line. The right duct, however, was greatly distended, filled with solid material, and occupied most of the space in the right sublumbar region (fig. 2). On careful dissection, parts of six yolks were encountered, ranging in distribution throughout the entire lumen. Since these yolk fragments appeared as definite concretions, surrounded by concentric layers of albumin, it was evident that they had remained within the duct for some time. Since the right ovary was absent, it would appear that these yolk concretions had arisen as ova within the left ovary and, following ovulation, had entered the ostium of the right duct. The free fimbriated extremity of the right duct, suspended by a part of the broad ligament, was an exact duplication of the normal left duct. Unfortunately, the continuity of either the right or the left duct with the cloaca could not be established, for in the preparation of the specimen for fixation and subsequent study, the cloaca, together with the posterior portion of both ducts, was accidentally removed. Both tubes were patent to within 1 cm. at least of the cloaca, and on the basis of the first case described, one might conclude that the right duct was patent throughout.

COMMENT

A review of the literature disclosed only a few cases in which the right oviduct attained a size equal to the left duct in the domestic fowl. Willier, in studying embryonic sex differentiation in the domestic fowl, in which various grafts were made on the chorio-allantoic membrane, found that rudiments of the right oviduct as well as of the right gonad were always present in varying amounts. These rudimentary ducts, however, were usually restricted to a cloacal stump and none was described as being functional. These observations

were corroborated by Domm, Crew, Brode, and Minoura in similar investigations of the domestic fowl, but the greater changes which were induced by these experiments occurred in the gonads, while the müllerian ducts were only slightly modified or were not affected. Crew and Fell described a case in which the right duct closely approximated the develop-

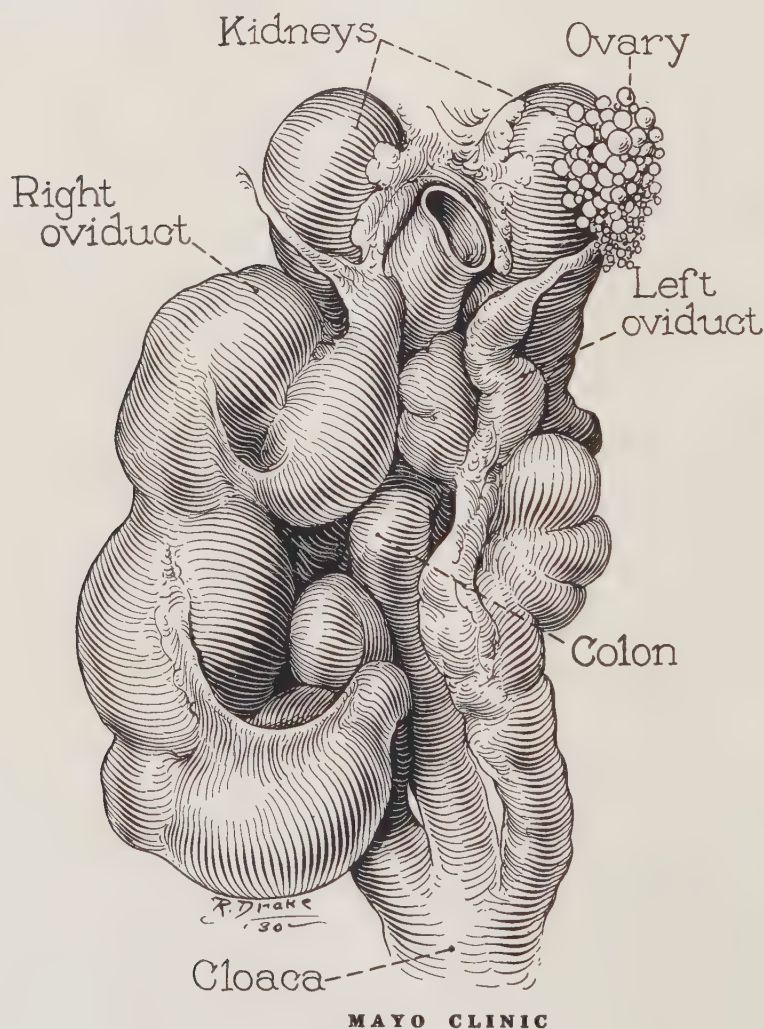


Fig. 2 Case 2. The persisting right oviduct filled with yolk concretions.

ment of the left. Kummerlöwe, in a comparative investigation of the gonadal system of female fowls, recognized two classes of birds, those in which there was normally no development of genitalia on the right side and a small group in which there was normally present a right gonad and oviduct as in certain species of hawks.

According to Kellicott, the müllerian ducts, which become the functional oviducts of maturity, appear about the fourth day of embryonic life as longitudinal invaginated bands of peritoneum. These invaginated bands which form grooves are situated lateral and parallel to the wolffian ducts. They gradually close, become tubular, extend posteriorly, and finally join the cloaca. The müllerian ducts ordinarily reach the cloaca during the seventh day, but do not open into it during embryonic life. Normal continuity between the two is established at the age of about six months. These ducts develop similarly in both sexes until about the eighth day, when they undergo retrogression in the male. Likewise, the right duct usually degenerates in the female. However, the stage at which the right duct in the female may start to degenerate into the remnant, which may persist into adult life (Willier and Yuh, '28), is extremely variable. Embryologically, then, there is no difficulty in accounting for the presence of a right duct in the mature hen, herein described, since, as Willier stated, both right and left ducts normally persist in varying degrees in the adult female.

Willier's explanation accounting for the persistence of the right müllerian duct or gonad, as shown by his experimental observations, is that the ova during early stages of incubation may be subjected to undue injury, such as low temperature or extreme agitation known to modify developmental processes. The incidence of typical structures in the genitalia of normally incubated eggs, according to Willier and Yuh, totals 18 per cent; whereas the eggs that were subjected to operative technic without grafting contained 24 per cent atypical structures and 34 per cent of the eggs which were hosts to grafts did not develop normally. This, as they have

concluded, shows that although grafting will increase the percentage of atypical structures, it does not specifically cause abnormal müllerian ducts, and that other factors, some of which are encountered in subjecting the egg to the operative procedure, may also enter into the formation of these ducts. Brode stated that atypical structures occurring at the site of the right ovary are determined to some extent by the existence or non-existence of primordial germ cells in the rudimentary ovary. It may be possible, then, that this rudimentary right ovary, which has been shown always to be present in varying degrees, may exert some power of inhibition or influence the degree of development of the right müllerian duct. Domm reported that in fowls from which the left ovary has been removed the amount of oviduct varies greatly, but in fowls in which bilateral oöphorectomy has been performed the amount of oviduct is greatly reduced. It would not seem, however, that there is any correlation between the size of the rudimentary right ovary and the right oviduct, for in the cases described the persisting right duct is equal in size to the normal left duct, although there was no macroscopic evidence of a persisting right ovary. The reverse is also true, as cases of persisting right ovaries cited in the literature tend to show that the rudimentary right duct does not necessarily develop on a parity with the persisting ovary. It would seem, then, that perhaps an endocrine secretion of some sort may induce the development of these rudimentary organs beyond their normal limits and may further act on either gonad or oviduct to the extent that they may become functional.

SUMMARY

Two cases of persisting right oviduct in the domestic fowl are reported. The right duct in one case was thought to be definitely functional, in that ova in various stages of degeneration were encountered in the lumen. One ovary which was in the normal position was present in each case.

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A PHOTOGRAPHIC METHOD OF ORIENTING SERIAL SECTIONS FOR RECONSTRUCTION

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FIVE FIGURES

The possibility of obtaining serial photographs of the surface of the paraffin block, during the process of cutting, in relation to fixed points for orientation purposes, occurred to the writer several years ago. Preliminary tests indicated that such a procedure is feasible, and during the past year it has been put into successful practice.

The first essential requirement consists in the vertical travel of the block, with its contained specimen, to a constant plane. This plane then, with its undisturbed potential section, is photographed in relation to notches in the aperture of a camera fixed over the microtome, before the section is cut from the block as is diagrammatically shown in figure 1. As the block is fed up, photographed, and cut, the lower planes of the object come to occupy the plane of the first section and so throughout the specimen. On the frame of each photograph there are thus established fixed points representing vertical axes through the specimen block which make it possible to superimpose the film frames and restore the original relationship. On completion of the series the serial film is finished for projection.¹

The serial sections are mounted in the usual manner and separately photographed at the selected magnification on

¹ The orientation film may be used to make a reconstruction directly and, if the draftsman is sufficiently careful in making his tracings, it will be entirely devoid of distortion.

bromide paper. When these serial bromide enlargements have been completed, the films made during the process of cutting are projected to the exact size of the enlarged bromides and each successive film frame is fitted into a master frame on an orientation board. By registering and pinning the bromides over the projected film images, the original relationship of the specimen to the orientation notches is

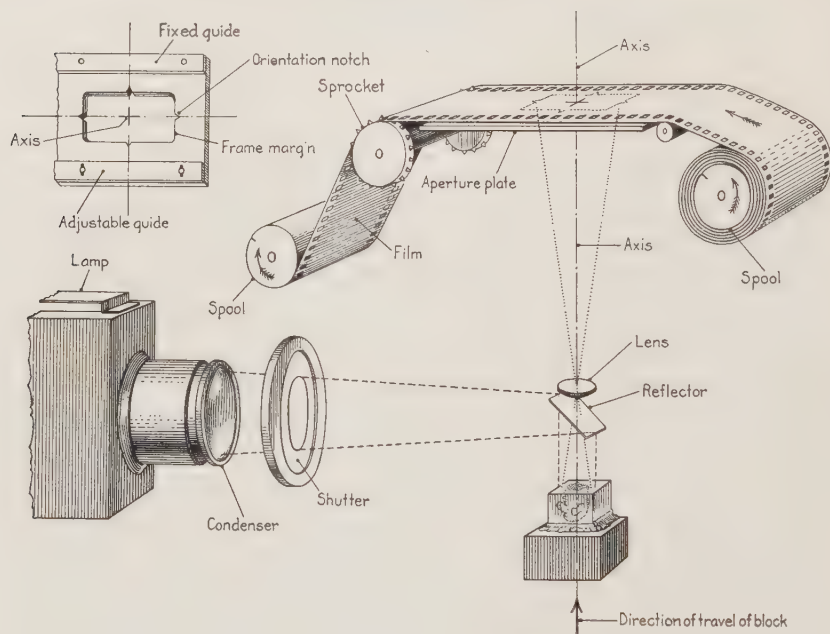


Fig. 1 Schematic drawing showing essential parts of the apparatus.

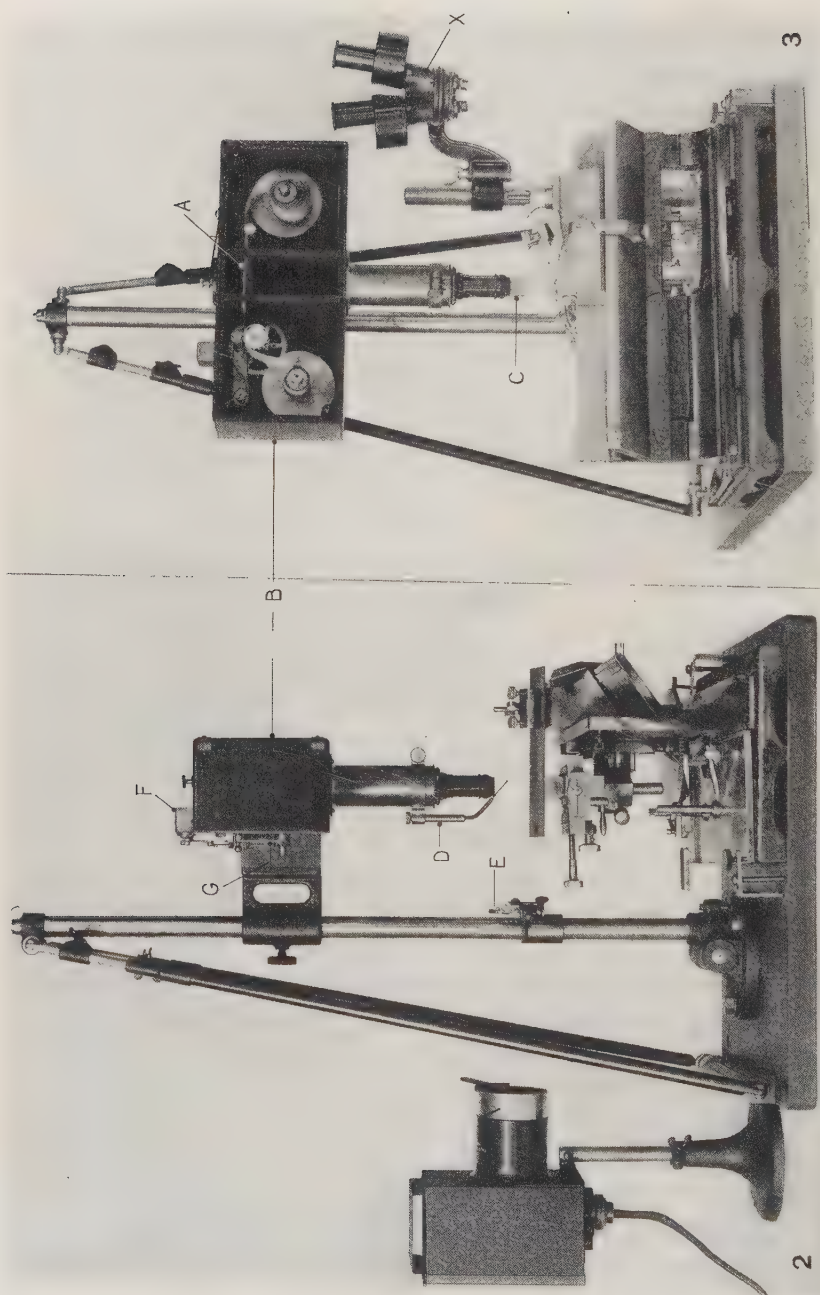
restored. Right-angle lines are then drawn on the bromides in relation to the orientation notches on the margin of each frame. The intersecting point of these lines represents a perpendicular axis through the specimen. These right-angle lines when projected through the block or reconstruction represent two planes intersecting at right angles and perpendicular to the plane of section. One is then prepared to make either a graphic reconstruction; wax model in positive, or preferably, a hollow wax mold into which plaster of Paris is

poured. From the latter a permanent rigid model is obtained which will not be distorted by changes in temperature—an advantage of much importance. Such, then, are the essential steps in the process. The required apparatus and technical procedure will now be described in detail.

EQUIPMENT

A rigid microtome with a vertical movement of the block is necessary. This should be checked for 'play' in the block guides and also for correctness of the ways with the feet of the base, which should be exactly 90° . A special camera (figs. 2 and 3) for carrying standard 35-mm. moving-picture film was constructed so that the aperture was double the standard size, i.e., 22 mm. $\times$ 36 mm. A rectangular aperture plate was made of brass and its margins filed with four V-shaped notches at right angles and opposite one another. These notches constitute the orientation points that register at each exposure. The length of the aperture is 2 mm. less than one-half the circumference of the sprocket that pulls the film over the plate. This dimension allows a space between each frame large enough for the V notches. The camera holds 80 feet of film (ample for 600 frames) of Eastman positive news stock. This film yields maximum contrast, is inexpensive and easy to work with. Two spools, a standard 1" sprocket wheel, two gears of equal size, a roller and a device for keeping the film taut and flat, as it is drawn over the aperture plate, constitute the chief features of the mechanism. The camera box was made of well-seasoned mahogany. A crank for turning the film one-half revolution with suitable stops for each exposure and a counter to record the number of frames complete the camera proper. The exposures range from one-tenth to four seconds and do not interrupt the rhythm of the cutting to an appreciable extent.

The lenses most suitable for use in the camera are either Zeiss planars or Bausch & Lomb micro tessars ranging in focal length from 16 mm. to 100 mm. Attention must be given to this detail, because much distortion can occur if uncorrected lenses are used.



Figs. 2 and 3 Two views of apparatus showing relationships of the essential parts. In figure 3 the side of the camera box is removed to show the mechanism. *A*, aperture plate; *B*, camera; *C*, cover-glass reflector; *D*, adjustable support for cover-glass reflector; *E*, small auxiliary condenser; *F*, counter; *G*, camera crank; *X*, binocular microscope available for examining knife and paraffin block.

The illuminant consists of a 6-volt, 108-watt, ribbon-filament lamp operating from a 110-volt A.C. current. The lamp house and condenser are regular laboratory equipment made by Bausch & Lomb Optical Company. A small auxiliary condenser $\times 3$ is used for the 16-mm. and 20-mm. lenses. The ribbon filament proves to be an ideal light source when used in conjunction with a cover-glass reflector to obtain vertical illumination. A shutter was placed in the path of the light beam in preference to combining it with the camera. This is not shown in figure 2, but was mounted on the front of the light condenser. It is operated by a bulb and rubber tube. The tube should be long enough to reach to the right of the apparatus if the 'water-on-the-knife method' is used when cutting. The vertical column supporting the camera was securely bolted down to a metal base and rigidly trussed with parts from a dismantled laboratory camera. The microtome was placed so that it could be clamped down to the cast-iron base and the clamps easily released for the purpose of moving the microtome with the block into proper alignment with the camera. A binocular microscope was also mounted on the metal base to facilitate the study of the surface of the block and also to determine when to begin photographing the surface of the block. The vertical column supporting the camera was hinged to the base so that the entire camera mount could be swung out of the way in case it should be found necessary to examine the surface of the block.

CUTTING AND PHOTOGRAPHING

The specimen to be cut is first stained in toto with alum cochineal and embedded in paraffin. Staining, however, is not necessary. The embedding and blocking of the specimen must be done so that a sufficient amount of paraffin is left around the specimen to give a block-surface area large enough to more than cover the film aperture and the V notches.

The manner of lighting the block from the illuminant and cover-glass reflector is found to be quite critical. Reflected light, that is, too oblique, is not good, since it shows too much

depth of the translucent block containing the specimen, and vertical illumination is therefore necessary. To secure this type of illumination the cover-glass reflector is usually set at 45° and works perfectly throughout the entire range of lenses used. It is found that the slightest change in the angle of the reflector obliterates the details of the surface of the paraffin block.

The thin sections are cut with the Huber 'water-on-the-knife method' described by Dr. C. H. Heuser.² The work of cutting is carried out in the dark-room, for the sake of convenience and accuracy, with safelights of sufficient brilliance that they do not register the translucency of the block and so spoil the definition and vertical illumination of the surface. The knife is examined under the microscope and if any small nicks are present they may be indicated and avoided. The knife is firmly clamped in the microtome and the paraffin block surfaced. Now the microtome is moved until the center of the object coincides with the optical axis of the camera and it is then clamped firmly to the metal base.

A lens is selected which covers the greatest surface area of the specimen and at the same time resolves the maximum amount of detail. The diaphragm is left wide open, since a constant plane is being recorded. The lens is focused while the light is being centered on the reflector. Next the reflector is adjusted to give totally reflected light. The effect of changing the reflector adjustment is best observed on a finely ground glass that is slipped over the film aperture. By moving the reflector and light source correct illumination is obtained. A hazy image or one that shows depth is unsatisfactory. When the details of the surface show clearly, one should tighten the reflector holder. It is now advisable to check all adjustments to see that the camera, light, microtome, and reflector are secure. This is imperative, for no subsequent movement is permissible after cutting is started. Any such movement would destroy the alignment and produce an imperfect vertical axis through the reconstruction.

² "Microscopical Technique," McClung, page 478, 1929.

The camera is loaded in position. Short strips of film are exposed and developed to find the proper time of exposure. Negatives of average density are preferable, since they show more distinctly when projected. It must be borne in mind that cutting is carried out under reduced light so that no stray light is permitted to fall on the block surface. Eastman Safe Light, series O A, with a 25-watt bulb, is ample to work by in comfort. Sectioning is begun on the blank block and several exposures are made to establish a cutting rhythm. The operator of the microtome handles the sections when using the dry method, while the camera and light shutter are controlled by a separate worker. It is possible for an individual to perform alone the entire operation of cutting and photographing, but this is not advisable when dealing with irreplaceable material. The reading of the counter is recorded when the first section is discernible. This facilitates the checking of the frames against the sections to more readily identify the frames. Before each section is cut an unexposed frame is moved into position and an exposure is made by opening the shutter for the required time. Now the section is cut and placed on either a tray or a slide, and these movements are repeated until the entire series is completed.

After completion of the series, the film is removed from the camera and developed for maximum contrast. The resulting negatives proved to be in several cases good enough for making out individual cells, though the flakiness of the paraffin somewhat marred the finer details (fig. 4). It will be noted that the details are sharp enough to make a reconstruction directly from such a negative. External form models made from such negatives would be without distortion and the model, if a wax mold is used, would represent the specimen as embedded.

ORIENTING

The serial sections are mounted in the usual manner and separately photographed at a selected magnification on bro-mide paper. This procedure greatly facilitates the making

of serial records on an enlarged scale, saves time, and produces a wealth of detail unobtainable in drawing. Incidentally, one source of error is reduced, thus increasing the precision of the model. The time-consuming practice of making serial drawings should be discarded. With the two serial photographic records that have been obtained in the above operations, one with orientation points, i.e., the film, and one without, we are now prepared to transfer the points from one to the other. It is with the serial bromides we are to make our model. This transferring is accomplished by projecting the orientation film with a 100-mm. lens so that its image superimposes over the bromide image. In other words, the enlargements are made to coincide.

To facilitate the holding of the film a special carrier is made and adapted to the Bausch & Lomb large photomicrographic apparatus. It consists of a brass plate with an aperture slightly larger than the film frame containing the image. Two guides are placed on the plate and separated the width of the film. A flat piece of a microscopic slide is cut 35 mm. $\times$ 75 mm. The edges of the slide are polished so that they do not scratch the film. Two springs are fastened to the plate to keep the glass plate holding the film flat against the plate. A suitable film spool support is added to the microscope to hold the roll of film.

A group of prints is selected and the corresponding frames of the film. By moving the projector to the proper distance from the projection surface the images are found to agree in general, but not perfectly. By comparing the bromide prints of 5μ sections with the projected frames of the films, a distortion, quite uniform and parallel to the travel of the knife, will be found to have occurred. As much as 10 per cent discrepancy in one direction has been measured, and to facilitate the accurate superimposing of the two images a compensating angle board was devised. This has proved to be quite satisfactory; it is arranged in the following manner (fig. 5).

A drawing board is hinged to the surface of a perpendicular support, and hinged so as to rotate around an axis parallel to the travel of the knife. The board is then tilted until the images agree. By so doing the projected image is elongated in one dimension. The depth of focus of the projection lens is sufficient that there is no appreciable loss of definition of the image, due to the tilting of the board. When this compensating angle is found, the board is fastened. A piece of

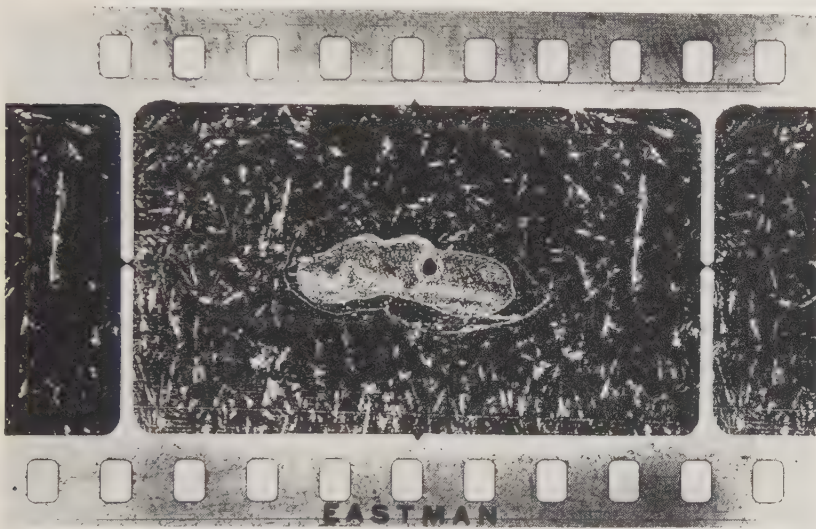


Fig. 4 Enlarged portions of film (standard, 35 mm.) showing one exposure of the block containing a pig embryo $\times 22.4$. Four notches can be seen on the margin of the frame, these to control orientation.

Bristol board is now tacked to the board and a broken outline of the frame carefully drawn with India ink. This outline with its V notches constitutes the master frame. The margin of the frame can be more readily seen in registering if the outline is broken. The arrangement of the board to the projector is shown in figure 5.

Another step is taken before the orientation lines are drawn. This consists in making a rapid survey through the series in order to establish a working median plane, provid-

ing the series is transverse. A piece of paper is placed over the master frame to protect it. Notches, corresponding to at least two of the V notches, are drawn on the paper. Rough outlines of random sections are drawn and the median planes ruled from selected sections. By surveying random sections and establishing this plane, which may be at quite an angle to the primary plane of the orienting notches, future work is made easier, especially for graphic reconstructions. When this mean plane is found, two pins are driven into the board on the line of the median plane beyond the margins of the

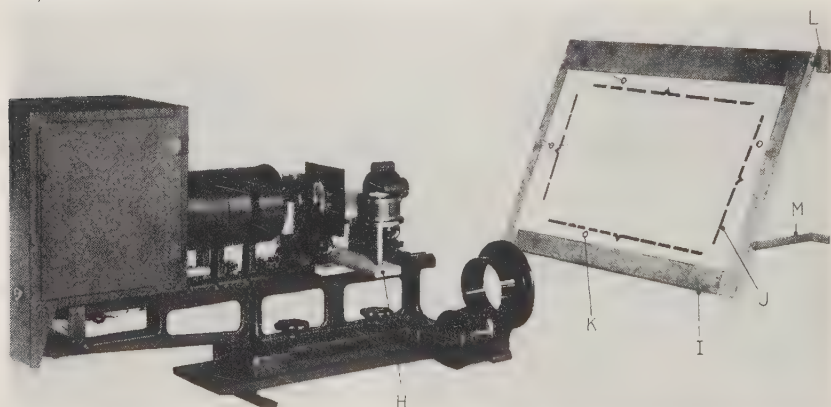


Fig. 5 Projector and adjustable projection boards. *H*, film support; *I*, adjustable projection board; *J*, master frame; *K*, one of the guide pins for ruling orientation lines; *L*, hinge; *M*, supporting arm.

master frame and two others at right angles (as is shown in fig. 5). Each frame is registered in the master frame on the orientation board and the corresponding bromide print, superimposed on the projected image. A straight edge held against the pins and a 6H pencil are used to rule the orientation lines. This work is done in the projection room, with a light-tight hood over the projector to exclude the stray light from the board. It is found that the orientation film, with an initial magnification of $\times 11.2$, when superimposed by projection to match serial bromides $\times 150$, yields an image adequate for registration of individual cells. From these

oriented serial records models are made that faithfully reproduce the form of the specimen as it was embedded.

It is indicated in the above that an appreciable distortion occurs in paraffin sections which is measurable. Whether this is brought about by the delicate serrations of the knife edge producing a corrugated effect in each section or is a jamming effect (more likely this factor) of the knife as it pushes through the paraffin has not been definitely determined. (It is feasible to correct distortions of the sections by the use of a prism when the records of the sections are made, providing the distortion is uniform. This can be checked against the film image.) It is well known that subsequent handling of the sections distorts them. In any event either of the above effects (serrations: jamming) would develop a disproportion in the sections at right angle to the travel of the knife. Because of these unavoidable distortions it is found necessary to devise a compensating board so that every part of the two images superimposes. It follows that the distortions inherent in the sections, if a prism has not been used in making the serial records, go into the reconstruction.

SUMMARY

The method here described provides a means of orienting serial sections by the aid of photography which is more exact than any heretofore available. In its use the factor of subjectivity is entirely avoided. Since reconstructions can be made directly from the presection record contained in the film, the distortions introduced by the microtome knife can be avoided; furthermore, should a section be injured in handling, its major details will have been preserved.

In conclusion, it may be advantageous to enumerate the essential equipment and technique employed. 1) A microtome with a vertical traveling block; 2) a camera equipped with a planar lens, carrying 35-mm. standard moving-picture film and having an aperture double the size of the standard frame, in which V-shaped orientation notches have been cut; 3) a cover-glass reflector to obtain vertical illumination;

4) uniform and constant light; 5) a shutter to time the exposure. All of the units are rigidly fastened together. For the orientation of the bromide photomicrographs, 6) a projector with a 100-mm. planar lens; 7) suitable film holder designed to prevent buckling of the film while being projected; and, finally, 8) a master frame—tilted if necessary—coinciding with the enlargement of the aperture and reconstruction.

I wish to express my deep appreciation to my fellow members of the staff of the Carnegie Embryological Laboratory, and especially to Dr. C. H. Heuser and Mr. G. O. Gey, for many helpful suggestions and stimulating interest in the perfecting of this technique.

CHANGES IN STATURE, WEIGHT, AND BODY BUILD OF FEMALE STUDENTS AT THE UNIVERSITY OF MINNESOTA DURING A PERIOD OF EIGHTEEN YEARS

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ONE FIGURE

In a recent investigation of the measurements of the women students entering the University of Minnesota during the year 1925-1926 (Jackson, '29), it was found that the index of body build averaged very low, even below that for the men. It was suspected that this very slender female physique might be artificial, due to 'dieting,' in conformity with recent fashions. In order to test this hypothesis, in the present study the corresponding data over a period of eighteen years have been compared. This comparison should demonstrate any marked change during the recent years when a slender female physique has been especially fashionable.

MATERIAL AND METHODS

The available records extend back to 1912. Since that time a complete physical examination has been required for all women entering the University of Minnesota. These examinations have all been made under the supervision of Dr. J. Anna Norris, the Director of the Department of Physical Education for Women. This gives assurance of a reasonable degree of uniformity in the technique of the measurements during this period. The methods used were described in detail in the previous study (Jackson, '29), and therefore need not be repeated here. The age was originally recorded in years and months. The measurements were taken without

clothing, except a thin costume (robe and bloomers). The weight of this costume (about 1 pound) was deducted in each case to obtain the net weight. The racial make-up is chiefly from Scandinavian sources, with Germans forming the next largest group, followed by those of British origin. Geographically, the student body is very definitely localized, predominantly from Minnesota and immediately adjoining states.

TABLE 1
*Average measurements of female students entering the University
of Minnesota, 1912 to 1929*

YEAR	NUMBER	AGE	WEIGHT	STATURE	CRUDE INDEX OF BODY BUILD
		<i>Years</i>	<i>Pounds</i>	<i>Inches</i>	
1912	400	20.358	119.438	63.508	29.61
1913	428	20.341	119.942	63.575	29.67
1914	520	20.608	120.606	63.706	29.71
1915	603	20.210	121.173	63.551	30.00
1916	695	20.629	121.241	63.651	29.92
1917	684	20.364	121.586	63.813	29.85
1918	681	20.077	120.400	63.719	29.65
1919	1047	19.966	120.375	63.639	29.72
1920	837	20.111	120.146	63.499	29.79
1921	976	20.469	121.675	63.699	29.98
1922	1017	20.067	121.576	63.731	29.93
1923	1095	20.254	121.669	63.564	30.11
1924	1123	20.114	121.347	63.799	29.81
1925	1204	20.012	120.249	63.699	29.63
1926	1366	20.010	120.212	63.714	29.61
1927	1378	19.534	120.987	63.843	29.68
1928	1339	19.994	122.104	63.891	29.91
1929	1393	20.081	121.789	63.580	30.12

There has been no marked change in racial make-up or geographical location during the entire period. The total number of individual records is 16,786, increasing from 400 in 1912 to 1393 in 1929 (table 1).

The data for the individual calendar years are shown in table 1, giving the number of cases and the arithmetic means for age, weight, and stature. The index of the body build in the last column is the 'crude' index, derived for each year by dividing the mean or average weight in pounds by the square

of the mean stature in inches. The quotient is then multiplied by 1000 to form a more convenient integer. This index of body build can easily be converted into the corresponding metric index through multiplication by the factor 0.07. This, of course, is not quite the equivalent of the true mean index, obtained from the individual indices, but the difference is not sufficiently great to invalidate comparisons for present purposes.

Furthermore, it should be noted that the probable errors indicated in table 2 are only approximate, and are obtained (for the means) through the usual formula—

$$\text{Probable error} = \frac{0.6745 \times \text{standard deviation}}{\sqrt{\text{number of cases}}}$$

assuming that in each year the standard deviations remained the same as those calculated for one year in the previous study (Jackson, '29). Since this assumption is not strictly correct, the probable errors are to this extent less dependable. Newcomer, however, found in Vassar students fairly constant standard deviations in weight, and especially in height, over a period of thirty-six years. The probable errors of the differences are calculated by taking the square root of the sum of the squared probable errors of the two numbers compared. Ordinarily, a difference is considered significant if it is at least three times the probable error of the difference, but in this case it is doubtless safer to require a difference to be four times its probable error in order to be considered as certainly significant.

OBSERVATIONS

Age

As shown by table 1 and the graph in figure 1, the mean age has fluctuated somewhat from year to year, but has in general declined from about 20.36 years in 1912 to 20.08 years in 1929. This represents a decrease of slightly more than one-fourth year in age. As shown by table 2, this difference for the two extreme years alone would be of somewhat doubtful significance, since it is only about twice its probable error.

If, however, we compare the mean age for the first six-year period with that for the last six-year period, or the first nine-year period with the last nine-year period, the differences are six to ten times their probable errors and are therefore certainly significant.

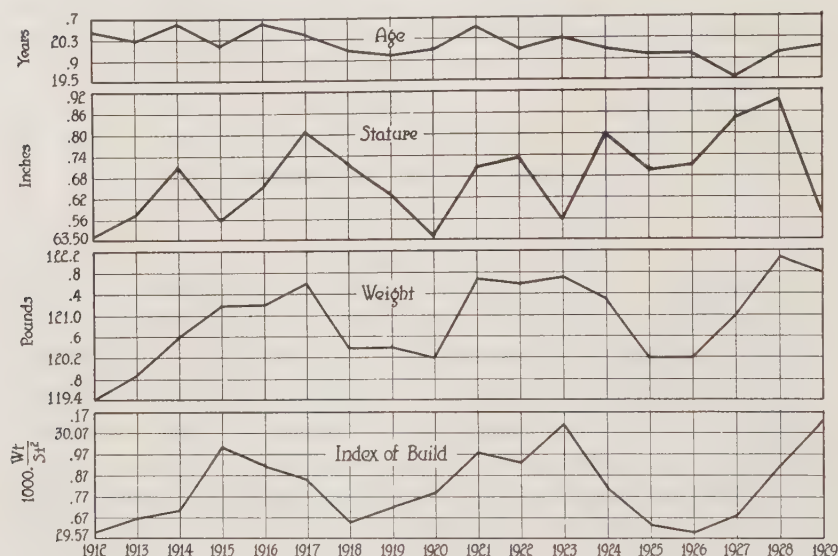


Fig. 1 Graphs showing the changes in mean age, stature, weight, and index of body build of the female entrants at the University of Minnesota from 1912 to 1929. The index of build is the crude index obtained by the ratio of the weight in pounds to the square of the stature in inches, multiplied by 1000 to give convenient integers.

It is thus quite clear that the age of entrance for the women students has been slightly but definitely lowered during this period. The same tendency appears even more clearly in the case of the male students at the University of Minnesota, as shown by MacKinnon and Jackson ('31). However, this change in age of the women is scarcely sufficient to affect appreciably the mean stature, weight, or weight-height index of body build.

Stature

The mean stature of the Minnesota women (table 1, fig. 1) has likewise varied from year to year, with a slight tendency to increase, especially in the latter half of the period. As shown in table 2, the average for the whole period is about 63.7 inches. There is, however, a rather marked decrease in

TABLE 2

Summary of changes in measurements of female students at the University of Minnesota. Weighted means and (approximate) probable errors for various periods

YEARS	NUMBER	AGE, YEARS	STATURE, INCHES	WEIGHT, POUNDS	CRUDE INDEX OF BODY BUILD
Whole period					
1912-29	16786	20.116±.018	63.694±.012	121.03±.09	29.829±.020
Comparing first year (1912) and last year (1929)					
1912	400	20.356±.114	63.508±.076	119.44±.59	29.614±.131
1929	1393	20.081±.061	63.580±.041	121.79±.32	30.127±.070
Difference		— .275±.129	.072±.086	2.35±.67	.513±.148
Comparing six-year periods					
1912-17	3330	20.426±.039	63.648±.026	120.82±.21	29.812±.046
1918-23	5653	20.162±.030	63.640±.020	121.04±.16	29.883±.035
1924-29	7803	19.951±.026	63.753±.017	121.13±.14	29.797±.030
Difference between 1912-17 and 1924-29		— .475±.047	.105±.031	.31±.25	— .015±.055
Comparing nine-year periods					
1912-20	5895	20.259±.030	63.633±.020	120.60±.16	29.774±.034
1921-29	10891	20.039±.022	63.727±.015	121.27±.12	29.859±.025
Difference		— .220±.037	.094±.025	.67±.19	.085±.043

1929. As a result, the difference in mean stature between 1912 and 1929 is insignificant, being slightly less than its probable error. The difference between the first and the last six-year period is more pronounced and probably significant, being over three times its probable error. The difference between the first half and the second half of the period is nearly four times its probable error, and therefore certainly significant.

We may therefore safely conclude that there has been a slight but significant increase of about $\frac{1}{10}$ inch in the mean stature of the female entrants during the period in question. For the male students, MacKinnon and Jackson ('31) found a larger increase of about $\frac{3}{4}$ inch during a longer period of about thirty years. European data for military conscripts similarly show a progressive increase in male stature in several countries for periods extending back over large portions of the nineteenth century.

The available data from other American colleges likewise indicate a definite increase in the mean stature of the female

TABLE 3
Crude index of body build for Vassar College students, grouped by five-year periods (from Newcomer's data)

YEARS	NUMBER OF CASES	MEAN BODY WEIGHT	MEAN STATURE	CRUDE INDEX Weight Stature ² $\times 1000$
		<i>Pounds</i>	<i>Inches</i>	
1884-1890	306	117.0	63.2	29.3
1891-1895	620	117.1	63.2	29.3
1896-1900	987 (990)	119.2	63.6	29.5
1901-1905	1164 (1172)	121.1	63.9	29.6
1906-1910	1268 (1269)	122.4	64.0	30.1
1911-1915	1474 (1481)	123.3	64.2	29.9
1916-1920	1233 (1234)	123.6	64.4	29.8

students. Newcomer ('21) published the annual data for Vassar College freshmen from 1884 to 1920, inclusive. Combining the first five years (on account of small numbers), the comparison shows an increase of 3.9 cm. (1.56 inches) in stature during this period. The arithmetic means for the five-year periods indicate a progressive increase since the second period (table 3). Mosher ('23) found a similar increase in the height of the female students in Smith College (1899 to 1921) and in Stanford University (1891 to 1921). She concluded that "Consideration of the average height of college women either separately in terms of the individual college and university group, or in terms of the measurements of the 21,383 examinations from Stanford, Vassar and Smith

combined, shows the same increase. We may therefore conclude that college women have increased in average height 1.2 inches or more in the last thirty years." Palmer ('29) also found an increase of nearly 0.6 inch in the mean stature of Hollins College freshman women between 1920 and 1927. The only discordant data are those of Gould ('29), who states that for Newcomb College (Tulane University) women from 1909 to 1924, "In the 16 years involved, there is no consistent upward or downward trend of freshman height or weight." However, in a later publication covering a period of twenty years (1909 to 1928), Gould ('30) finds an increase of 0.26 inch in mean stature, comparing the last nine years with the first eleven years.

Weight

The mean body weight of the Minnesota women students (table 1, fig. 1) has varied somewhat during the eighteen-year period, but, like the stature, it shows a general tendency to increase. As shown in table 2, the average for the whole period is about 121 pounds. Comparing the first with the last year shows an increase of 2.35 pounds, which is 3.5 times its probable error and therefore probably significant. The fluctuations are such that the averages for the three six-year periods are nearly the same, showing only an insignificant increase. Comparing the nine-year periods, however, shows an increase of 0.67 pound, which is nearly four times its probable error and therefore almost certainly significant.

We may therefore conclude that there has been a slight but significant increase of at least two-thirds of a pound in the mean weight of the female entrants at Minnesota during the period in question. For the male students, the increase in weight (like that in height) has apparently been greater, amounting to 3.9 pounds over a thirty-year period (MacKinnon and Jackson).

For the women in other colleges, the data available for comparison are less extensive for weight than for height. Newcomer ('21), however, gave the mean annual weights for

the Vassar freshmen from 1884 to 1920, inclusive. Again combining the first five years, there is an apparent increase of 4.4 pounds in average weight during this period. The means for seven five-year periods show a progressive increase from 118.1 pounds in the first to 124.7 pounds in the last period, a gain of 6.6 pounds. In table 3 the means have been corrected by deducting 1.1 pounds for clothing. Palmer ('29), on the other hand, in the much smaller group of Hollins College freshmen, found between 1920 and 1927 an irregular decrease, amounting (comparing first and last years) to about 3.4 pounds. However, this decrease is statistically of doubtful significance. Gould ('29, '30) found no consistent change in the trend of weight in the Newcomb College freshmen between 1909 and 1928.

Body build

The index of body build used in the present study is the weight:height² ratio. The crude index (table 1, fig. 1) has varied somewhat from year to year, tending in general to follow the curve for body weight. As shown in table 2, the arithmetic mean for the whole period is about 29.8, indicating a relatively slender physique. A comparison of the index for the first with that for the last year (table 2) indicates a slight increase of 0.513, which is over three times its probable error and might therefore be considered as probably significant. However, a comparison of the six-year or the nine-year periods reveals no significant change in the index. It may be concluded that there has probably been no appreciable change in body build of the female students at Minnesota during the period in question. There has certainly been no decrease in the index; if any change, it is more probably in the direction of an increase. MacKinnon and Jackson ('31) likewise found little change in the body build of the male students at the University of Minnesota over a period of thirty years.

For additional comparison, the crude indices of body build for the Vassar students, grouped by five-year periods, have been calculated from Newcomer's tables II and IV, deducting

1.1 pounds for the weight of the examining robe. The results are shown in table 3. It is clear that between 1884 and 1920 the Vassar students, although increasing in both weight and stature, have undergone relatively slight changes in body build, as measured by the crude weight: height² index. There is apparently a slight increase in the index from 29.3 in the periods 1884-1890 and 1891-1895 to 30.1 in the period 1906-1910. The slight decrease to 29.9 in 1911-1915 and to 29.8 in the latest period, 1916-1920, is of doubtful significance.

In general, the Vassar students are evidently slender in physique, like those at Minnesota. The Hollins College freshmen are even more slender, the crude index of body build for the seven-year period (1920-1927) being only 28.36. On account of the relatively small numbers, the data for the individual years are of doubtful significance. Palmer ('29) found that the proportion of apparent 'underweights' in this group increased up to 1925, then decreased to 1927. She states that "Until more data are available on the trend of weights of women students in other colleges, we shall be unable to draw conclusions concerning the influence of style changes in college women's weights in recent years. The impression of the public appears to be that this influence has been considerable." Gould ('30) likewise noted a recent tendency toward a lower weight-height index in the Newcomb College students, apparently due to increasing stature with nearly stationary body weight.

SUMMARY

1. The measurements included 16,786 individuals, ranging from 400 in 1912 to 1393 in 1929. There has been a decrease of about one-fourth year in the mean age of the female entrants to the University of Minnesota during this period of eighteen years.

2. The mean stature shows fluctuations for the individual years, with a slight but significant increase of about $\frac{1}{10}$ inch during this period.

3. The mean body weight is likewise variable, with an apparent increase of 2.35 pounds between the first and the last year of the period. If the six-year or the nine-year periods are compared, however, the increase is much smaller.

4. This progressive increase in stature and weight is in general agreement with the data for female entrants in most other colleges, and also with the corresponding data for young men.

5. The changes in body build (measured by the weight: height² index) of the female students are much less marked, although there is a slight tendency toward an increase (stouter body build) evident in both the Minnesota and the Vassar data. On the whole, the evidence does not support the theory of an artificial decrease in body weight of college women to conform with female fashions in recent years.

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NEW SILVER METHODS FOR PARAFFIN SECTIONS

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ONE PLATE (FIVE FIGURES)

With the constant increase in the use of silver stains for the demonstration of nerve cells and their processes, the classical methods of Bielschowsky and of Cajal have become the standard neurological techniques for material done in bulk. To obtain the best results on pieces of tissue impregnated in toto with silver solutions, the block of tissue must be very small so that the parts farthest removed from the surface may be properly impregnated without overimpregnation of the more superficial regions. The desirability of using large blocks of organs and tissues for anatomical and pathological work and entire embryos in embryological work has led to many attempts to obtain a dependable silver method for sectioned material.

Methods suitable for frozen sections have been devised by Bielschowsky, O. Schultze, Laidlaw, and others, but less success has been attained with paraffin sections. In my experience, the techniques suitable for frozen sections cannot be applied to paraffin sections without modification. Several methods for paraffin material have been described, the most recent by Kernohan. These existing methods did not satisfy certain requirements. In many types of research it is necessary to obtain constantly in serial sections well-stained nervous elements including cells, fibers, and terminations and at the same time leave the connective tissue colorless or very lightly stained. This need of differentiating between connective tissue and nerve fibers led the writer to experiment

with many possible types of silver stains. Of this number, several of the formulae gave good results consistently. Without reviewing the literature or discussing the theoretical and chemical aspects of silver stains, the present paper merely records such methods as may possibly be of value to other workers.¹

METHODS

Formula A

1. Fixation: 10 to 20 per cent commercial formol or Bouin's picro-aceto-formol for seven days or longer.

2. For formol material: wash in running water one day or more. After Bouin's fixation, transfer to 70 per cent alcohol—many changes of 70 per cent alcohol until most of the picric acid disappears.

3. Dehydrate; in the dehydration 1 cc. of ammonium hydrate should be added to every 30 cc. of 80 per cent and 90 per cent alcohols, in each of which the tissue should remain at least twenty-four hours. Absolute alcohol, two to four hours (change once).

4. Clear in chloroform or cedar oil.

5. Embed in paraffin.

6. Cut sections from 2 to 40 μ or more.

7. Mount with albumen fixative or by Masson's formol-gelatin method.

8. Remove paraffin with xylol.

9. Absolute alcohol—several minutes.

10. Twelve hours or more in 95 per cent alcohol to which 2 cc. of ammonium hydrate has been added to every 100 cc. of 95 per cent alcohol.

11. Rinse in 80 per cent alcohol.

12. Transfer to 40 per cent silver-nitrate solution for twenty minutes or longer; several hours, days, or weeks does no harm so long as the sections are kept in the dark.

13. Rinse quickly with distilled water.

14. Cover the sections with 20 per cent formol for two to five minutes; several hours does no harm.

15. Drain formol off the slide, but do not wash.

16. Cover slides with diammoniacal silver solution² made as follows: To 4 cc. of 20 per cent silver nitrate add several drops of ammonium

¹I wish to express my appreciation to Dr. G. F. Laidlaw, Department of Surgery, College of Physicians and Surgeons, for valuable suggestions, especially as regards the use of 40 per cent silver nitrate instead of weaker solutions.

²Heat the diammoniacal silver solution to 30° to 55°C. before covering the sections with it. This is the optimum temperature at which the reduction of the silver occurs in the nervous elements.

hydrate to precipitate the silver. Then, with constant shaking, continue adding ammonium hydrate drop by drop until the precipitate is redissolved. Following this, add one drop of ammonium hydrate to every 2 cc. of the original 20 per cent solution of silver nitrate. To this add 4 cc. of distilled water. Allow this solution to remain on slides until sections turn a yellowish brown or as soon as the nerve fibers have been stained. This may be controlled under the microscope.

17. Rinse in distilled water—approximately one minute.

18. Tone with solution of gold chloride made as follows: 1 gram gold chloride to 300 cc. distilled water. Add ten drops of glacial acetic acid to every 50 cc. of gold-chloride solution. Allow sections to stay in the toning solution for ten to fifteen minutes. The acetic acid bleaches the connective tissue.

19. Wash with distilled water.

20. Fix in 5 per cent sodium hyposulphite for about five minutes.

21. Wash in running water or in several changes of water on the slide.

22. Dehydrate; clear in carbo-xylol, then xylol, and mount in balsam.

Comment

Step no. 1. Formol may be neutral or acid—either gives good results.

Step no. 15. Caution! Drain off formol, but never rinse it off—to do so interferes with the subsequent reduction of the silver in step no. 16.

Step no. 16. It is essential to shake the solution constantly while clearing the silver precipitate. This solution may be kept for several days or a week and still give good results.

Step no. 17. After rinsing with water, if examination under the microscope shows that the sections are too heavily stained, 4 per cent acetic acid may be used to decolorize them to the desired intensity.

Step no. 19. After washing in distilled water, if the sections, on examination under the microscope, are too light, they may be darkened by treating with 4 per cent oxalic acid, which reduces the gold.

Step no. 20. Wash out the hypo well before dehydrating. Failure to do so frequently causes stains on the sections.

Formula B

Preliminary steps, 1 to 11, are same as in formula A.

12. Transfer to 40 per cent silver-nitrate solution for ten minutes or more.

13. Rinse quickly with distilled water.
14. Cover the sections with 20 per cent formol for two to five minutes.
15. Drain formol from slide, but do not wash.
16. Cover slide with diammoniacal silver solution, made as in formula A, for ten to thirty seconds.
17. Reduce silver with 20 per cent formol for four or five minutes—or until section turns a gray or brown color—then rinse in distilled water.
- Remaining steps (18 on) as in formula A.

Formula C (long method)

Preliminary steps, 1 to 11, same as formulae A and B.

12. Transfer to 40 per cent silver-nitrate solution for three to five weeks for nerve endings; five to six weeks, for cord and ganglia. Keep in the dark. The silver is partially reduced by this prolonged treatment of the tissues with strong silver nitrate. The sections are stained brown or gray. The nerves appear gray or black when examined under the microscope.

13. Reduction is completed by treatment with 20 per cent formol for five to ten minutes.

14. Rinse in distilled water.

15. Tone as usual—see formulae A and B.

16. If a purple background is desired, reduce the gold with 1 per cent oxalic acid until the section becomes a faint violet color; if a colorless background is desired, eliminate step 16.

17. Hypo—five to twenty minutes—longer does no harm. Wash, dehydrate, clear, and mount as in formulae A and B.

Formula D (long method)

Steps 1 to 9 same as in formulae A, B, and C.

10. Run down through the alcohol series, i.e., 95, 80, 70 per cent, to distilled water—then leave for thirty minutes in 0.5 to 1 per cent aqueous solution of acetic acid.

11. Rinse several times in distilled water.

12. Transfer to 40 per cent silver solution: Five to six weeks for motor end plates, six to seven weeks for cord and ganglia.

Remaining steps (13 on) as in formula C.

In each of the four techniques, nervous elements including fibrillae and terminations are stained black. The most satisfactory results for amphibian material have been obtained with formula A. All four of the formulae give equally good

results for mammalian tissues. If it is desirable to stain the connective-tissue elements, a cytoplasmic counterstain may be superimposed on any of the silver stains after they have been thoroughly washed in water, following the treatment with hypo.

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PLATE 1

EXPLANATION OF FIGURES

1 From a photomicrograph of a transverse section through the spinal cord of a cat, showing a large motor cell. Surrounding this cell are a number of axons of varying sizes, running in many directions in the gray matter. The upper part of the figure shows a number of large and small axons of the white matter cut transversely. $\times 548$. Technique by formula B. Fixation in formol.

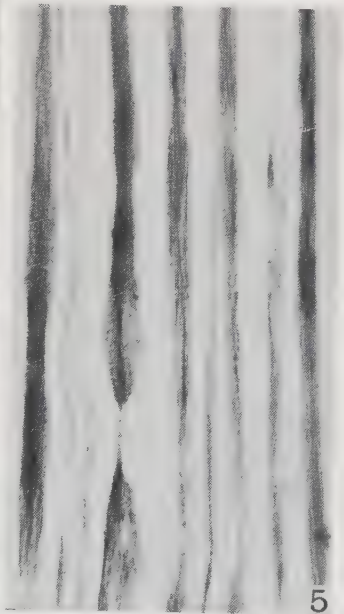
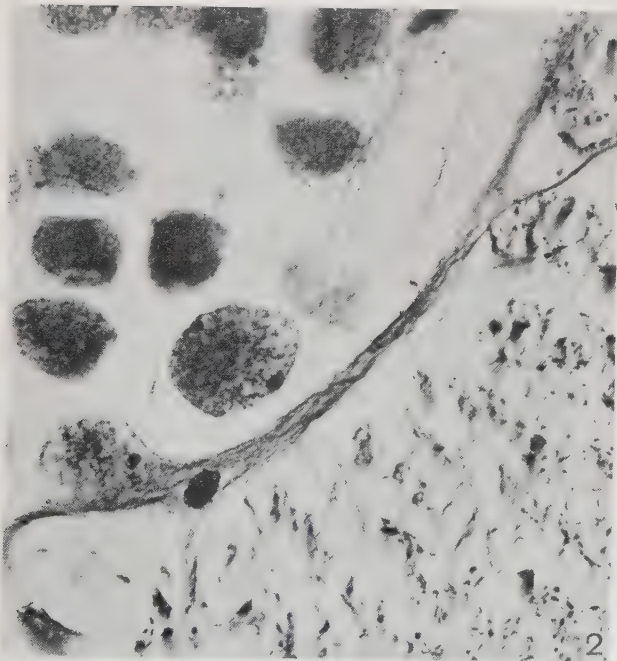
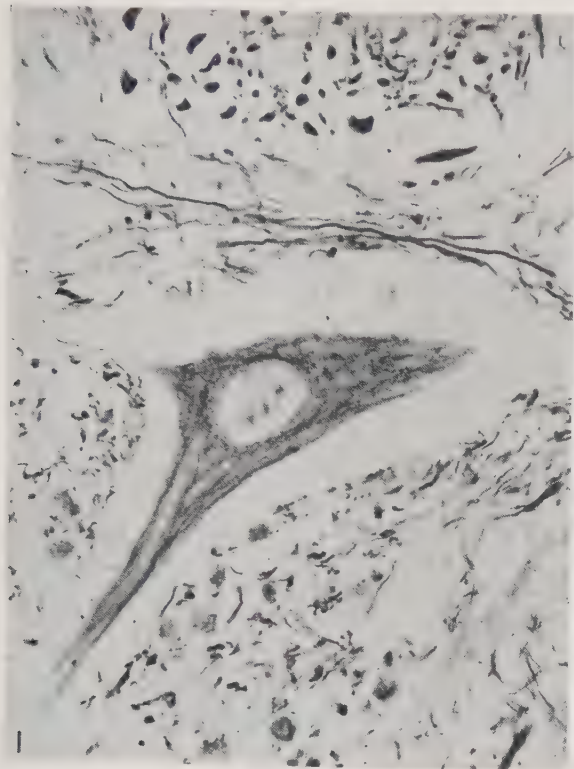
2 From a photomicrograph of a part of a transverse section through the spinal cord of a 35-mm. *Amblystoma punctatum* larva. A large motor cell is shown with its axon, dendrites, and the large pigment spot which is so characteristic of this type of cell. $\times 929$. Technique by formula A. Fixation in Bouin's fluid.

3 From a photomicrograph of a section of intercostal muscle of a cat, showing an oblique section of a nerve fiber containing several axons. Two of these axons are shown in longitudinal section, one in its entirety and terminating in the ramifications of a motor end plate. $\times 548$. Technique by formula B. Fixation in Bouin's fluid.

4 From a photomicrograph of a transverse section through three nerves of a 30-mm. *Amblystoma punctatum* larva; note the large and small axons. $\times 196$. Technique by formula B. Fixation in Bouin's fluid.

5 From a photomicrograph of a longitudinal section of the sciatic nerve of a cat, showing the neurofibrillar network of the axons. One node of Ranvier is included. Note the pencilling of the axon at the node. The neurilemma is shown faintly. $\times 548$. Technique by formula B. Fixation in Bouin's fluid.

None of the photomicrographs have been retouched.



THE LATER DEVELOPMENT OF THE SINUS
VENOSUS AND THE RELATION OF THE
SINO-ATRIAL NODE TO IT, IN
THE CALF HEART

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ONE PLATE (FOUR FIGURES)

Stiénon ('27) and Shaner ('29), two recent writers on the development of the sino-atrial node, have doubted that the node is really a remnant of the sinus venosus, and have suggested that it may belong to the cavosinal junction. Doctor Shaner suggested to me the desirability of a reinvestigation of the development of this part of the heart to determine the relation of the node to the sinus venosus.

The study was carried out on calf material. The history of the sinus venosus in this animal departs somewhat from that given by Morrill ('16) and others. A short account of such departures and the answer to the main question make up this communication.

EMBRYOS UNDER 30 MM.

The sinus venosus in early calf embryos shows nothing unusual. In the 20-mm. embryo a set of muscle ridges radiate beneath the dome of the right atrium from the right sinus valve and the septum spurium. Such ridges are the first trace of the crista terminalis. In this and subsequent stages of the calf, the crista is fused with the right valve and can be taken to mark the sino-atrial boundary when the valve disappears. The condition in the calf is the same as in man (Tandler, '12). Morrill records an interval between the valve and the crista terminalis in the pig.

EMBRYOS OF 30 TO 60 MM.

In embryos of from 30 to 60 mm. the sinus venosus and right atrium develop several peculiar features. The right sinus horn lengthens out, so that in a 60-mm. embryo (fig. 1) it has the shape of a flattened hourglass. The two valves are unusually long and the septum spurium correspondingly insignificant. The upper and lower expanded portions of the sinus receive the superior vena cava and the inferior vena cava together with the coronary sinus, respectively. Between the expanded portions is a very much constricted middle section (figs. 1 and 2) which is a shallow groove marked off from the atrium by the valves only. The lower third of the right valve is very broad; the left valve is deeply notched and already vestigial.

There is no clear trace of a septum secundum in a 30-mm. embryo. This structure seems to arise late in the calf heart. In the 60-mm. embryo the septum is a well-developed muscle ridge that begins on the anterior wall of the right atrium just above the point where the free edges of the sinus valves meet it, and then arches upward and backward over the foramen ovale to join the interatrial septum (figs. 1 and 2). At this point the septum secundum loses its separate identity. It is continuous, however, with a muscle ridge that lies behind the valvulo-interseptal space and follows the left sinus valve well down along the middle segment of the sinus venosus (figs. 1 and 2). The two portions make a horseshoe-shaped arch across the foramen ovale.

The septum secundum of the calf heart offers one more variation of this much discussed structure. There is no doubt of its existence once it appears. It is a definite muscle ridge and not an infolding of the atrial wall. It has nothing to do with the septum spurium and the crista terminalis, both of which lie above and apart from the septum secundum.

EMBRYOS OF 60 TO 90 MM.

In further growth of the calf embryo the septum secundum expands greatly. Its free edge contracts, so that in a 90-mm.

embryo it narrows the interatrial communication and hides the foramen ovale (fig. 3). The septum as a whole is greatly thickened and enlarged, so that its posterior limb lifts the left sinus valve from the heart wall.

During the same period the crista terminalis enlarges greatly and comes to bear upon itself the atrophied right sinus valve (fig. 3).

Of the two sinus valves, the left one is obliterated in the 90-mm. embryo heart except for a short stretch below the crista terminalis. The right valve is a slender C-shaped structure still traceable throughout its former extent. The upper part of the right valve now lies upon the inner surface of the crista terminalis. This portion of the right valve together with the remnant of the left one guards the entrance of the superior vena cava. The middle and lower thirds of the right valve guard the orifices of the inferior vena cava and the coronary sinus.

In subsequent development the two sinus valves disappear. They are not present as any obvious gross structures in the adult beef heart. Obscure muscle bands beneath the pericardium probably represent them here as in other forms.

POSITION OF THE SINUS NODE

As has been recently brought out by Shaner ('29), the sino-atrial node becomes unmistakable in calf embryos of around 100 mm. The node is present in the 90-mm.-embryo heart above described. Its position is shown in figure 4. The node lies on the ventral wall of the superior vena cava just above the line along which the vein plunges into the heart. The nodal tissue is in histological continuity with the crista terminalis to the right and with the interatrial muscle band of Papez to the left. The whole structure lies just above the septum spurium. All sino-atrial landmarks are still clear in this heart. The node lies just above them and in closest relation to sinus and atrial structures. The sinus node can therefore be assigned to the sino-atrial junction.

It may be pointed out in passing that the sinus node in the calf heart abuts against the ventromedial surface of the superior vena cava, not the ventrolateral, where Keith and Tandler place it in man.

A grant from the Banting Research Foundation made possible the completion of this study. It is a pleasure to record my acknowledgments to Profs. D. G. Revell and R. F. Shaner for helpful advice and criticism and to Mr. A. G. Fairall for the preparation of the material used in this study.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 Model of the atria and sinus venosus of the heart of a 60-mm. calf embryo. The ventral atrial wall has been largely cut away to display the sinus venosus as seen from the interior of the right atrium. $\times 20$.

2 Section through heart of the 60-mm. calf embryo shown in figure 1 at level indicated in that figure. The section shows the narrowest part of the sinus venosus and the two roots of the septum secundum. $\times 10$.

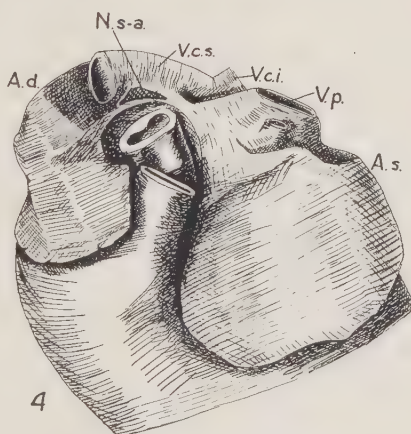
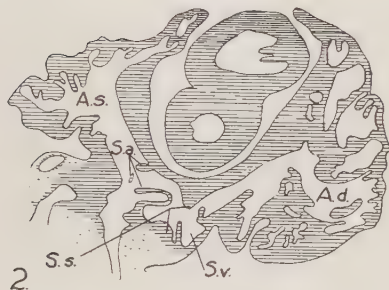
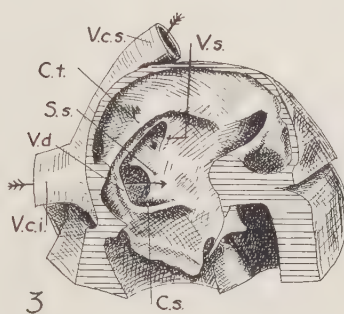
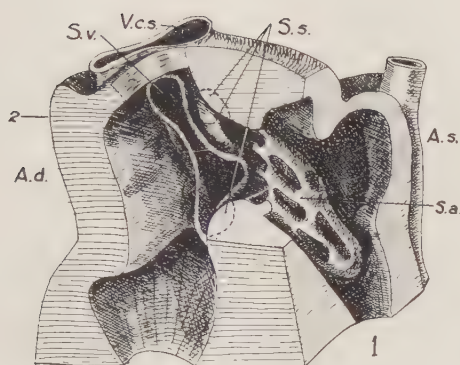
3 Model of the interior of the right atrium of a 90-mm. calf embryo. To show the extent and relations of the septum secundum, crista terminalis, and the sino-atrial valves. $\times 20$.

4 External and ventral view of the same model as in figure 3. To show location of the sino-atrial node. $\times 20$.

ABBREVIATIONS

A.d., s., right and left atrium
C.s., coronary sinus
C.t., crista terminalis
N.s-a., sino-atrial node
S.a., atrial septum
S.s., septum secundum

S.v., sinus venosus
V.c.s., i., superior and inferior vena
cava
V.d., s., right and left ventricle
V.p., pulmonary vein



THE TRANSPLANTATION OF MOUSE OVARIES INTO THE RAT

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In the course of certain attempts to obtain a cross between rats and mice, transplantations of mouse ovaries were made into forty female rats. After the rats were anaesthetized, a single dorsal incision, carefully made, permitted the observation of first one and then the second ovary. The rats operated on were from thirty to forty-five days of age and possessed rather small ovaries. Accordingly, the excision of the ovaries was made under a binocular microscope, and care was taken to remove as completely as possible all ovarian tissue. One, sometimes two, mouse ovaries were substituted for each rat ovary removed. The transplanted ovaries were placed in the ovarian capsules of the rat, but no attempt was made to sew up the opened capsule. It was therefore quite possible that the transplanted ovaries might have subsequently fallen out of the capsule, especially as the transplanted ovaries were taken from three- to four-week-old young mice and were consequently quite small.

The operated animals were kept from one and one-half to two and one-half months after the operation. Vaginal smears were taken from time to time, beginning about one month after the operation. Thus sufficient time was allowed for the transplants to 'take.' The presence of oestrous smears was considered to be an indication that functional ovarian tissue was present. Despite the fact that in many of the females there was no vaginal evidence of an oestrous cycle, all of them were kept continuously with male rats for at least one month before they were killed. Mating unques-

tionably occurred in a number of instances, as the presence of vaginal plugs attested. In only one case were young produced, and these were typical low-grade hooded rats from a low-grade hooded female (no. 32) mated to a low-grade hooded male. This female had received the ovaries of an intensely black mouse, and if the young were rat-mouse hybrids, one would expect them to be self-colored or at least very high-grade hooded. Further breeding tests showed them to be undoubtedly rats.

Since it became necessary for various reasons to terminate the experiment, the operated females were killed in the course of a week and autopsies performed in most cases. The results are given in table 1.

Judging from the vaginal smears, one-half (twenty out of forty) of the operated animals contained functional ovarian tissue at from seven weeks to ten weeks after the operation. It is not certain that in every case the ovarian tissue was mouse tissue. According to Haterius ('28), a very small unexcised ovarian fragment is capable of a considerable degree of regeneration in a short period. The technique of operation was essentially that employed by Haterius on mice, that is, the ovary was cut off at or below the hilus. About 10 per cent of regeneration was observed in his experiments.¹ Davenport ('25) has reported as high as 64 per cent regeneration of ovarian tissue, but his experimental period was longer than that of these experiments, and doubt is not completely removed that thorough extirpation was practiced. The case of rat no. 32 in these experiments proves that in her case functional ovarian tissue was proliferated after the operation, for she produced a litter of normal rats.

That some of the twenty rats that showed functional ovarian tissue contained mouse ovarian tissue is indicated by the fact that in a number of instances the only ovarian tissue found at autopsy was located far from the site of the original ovaries. In two cases such tissue was found growing on the tubal walls, in two other cases ovarian tissue was found grow-

¹ Pencharz ('29) reports less than 3 per cent.

TABLE 1

Findings on forty female rats that had received transplanted mouse ovaries

NO.	VAGINAL INDICATIONS	AUTOPSY FINDINGS	STAGE OF VAGINAL CYCLE AT AUTOPSY	DATE OF TRANS-PLANTA-TION	DATE OF DEATH
2	Oestrus	Ovarian tissue on both sides	Beginning of oestrus	1/11	3/24
3	Oestrus	Ovarian tissue on both sides	Before beginning of oestrus	1/13	3/24
4	Oestrus	Dead tubal embryo and ovarian tissue on right	Before beginning of oestrus	1/13	3/27
5	Oestrus	Pseudopregnant	No autopsy	1/16	3/27
6	Oestrus	Ovarian tissue on left omentum	Before beginning of dioestrus	1/16	3/23
7	Oestrus	Slight ovarian tissue on right	Before beginning of dioestrus	1/19	3/27
8	Oestrus	No ovarian tissue found	During dioestrus	1/19	3/23
9	Almost continuous oestrus	Ovarian tissue on both sides	During oestrus	1/19	3/27
10	No oestrus	No autopsy	Castrate smear	1/19	3/28
11	No oestrus	No ovarian tissue	Castrate smear	1/19	3/28
12	Oestrus	No ovarian tissue found	Before beginning of dioestrus	1/20	3/27
13	No oestrus	No ovarian tissue	Castrate smear	1/20	3/28
14	Oestrus	Ovarian tissue on left tubes	Just before dioestrus	1/20	3/25
15	Oestrus	Ovarian tissue on both sides	Just before dioestrus	1/20	3/24
16	No oestrus	No ovarian tissue	Castrate smear	1/20	3/28
17	Oestrus	Ovarian mass on right	During metoestrus	1/23	3/27
18	Oestrus	Slight ovarian mass on left	During metoestrus	1/23	3/27
19 to 22	No oestrus	No ovarian tissue	Castrate smear	1/23 to 1/25	3/28
23	Oestrus	Large ovary on right	Just before dioestrus	1/25	3/24
24	No oestrus	No autopsy	Castrate smear	1/25	3/28
25	Oestrus	Ovarian tissue on right	During dioestrus	1/25	3/27
26	Continuous oestrus	Ovary on left	During oestrus	1/25	3/27
27	Oestrus	Ovarian tissue on both sides	During dioestrus	2/1	3/23
29	No oestrus	No autopsy	Castrate smear	2/1	3/29
30	No oestrus	No ovarian tissue	Castrate smear	2/1	3/29
31	Oestrus	Ovarian tissue on right tubes	During dioestrus	2/1	3/23
32 ¹	Oestrus	Ovary on left		2/1	4/29
35	No oestrus	No autopsy	Castrate smear	2/6	3/29
37 to 43	No oestrus	No ovarian tissue	Castrate smear	2/10	3/30

¹ Gave birth to four young rats.

ing on the body wall, and in other cases tissue was found in the fat-bodies in abnormal locations. Again in two cases where no ovarian tissue was found the oestral changes of the vaginal epithelium indicated that some must have been present. The missing tissue must have been located inconspicuously some distance from the ovarian site, for the latter was always very carefully examined. As mentioned previously, the transplanted mouse ovaries might very well have fallen out of the capsule onto other sites.

Furthermore, most of the ovarian tissue observed at autopsy resembled typical heterotransplant tissue, in that there was marked overgrowth of connective tissue and lymphocyte infiltration. It is therefore evident that mouse ovaries transplanted into ovariectomized rats are capable of surviving and carrying on oestral function after a period of as long as ten weeks and probably longer.

The cases of rats nos. 9 and 26 are worthy of note in that the vaginal smears indicated an almost continuous oestrous state, for a true dioestrous smear was never observed and only occasionally a metoestrous smear. Is it possible that they contained functional mouse *and* rat tissues which were not synchronous in oestrin production?²

It is realized that the results of these experiments are indicative rather than conclusive. It may be pointed out, therefore, that the persistence of mouse ovarian tissue in the rat encourages the hope for follicular function and ovulation.

The writer is indebted to Prof. W. E. Castle for providing facilities for this investigation and for assistance in the operations performed.

² Similar vaginal indications have been reported by Castilio ('28) on regrafting ovaries into castrated females, by Drips ('29) in rats with very small ovarian fragments, and by Matsuba ('29) in the normal female in a parabiotic union with a castrate male or female.

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THE SIZES OF FERRET PRONUCLEI

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INTRODUCTION

Almost everywhere throughout the literature on pronuclear development are found general references to size. The two pronuclei are stated to be of different size and their changes in size are mentioned, but beyond this, with regard to mammalian pronuclei, very little exact work has been carried out. In those records where measurements have been recorded, they have not been analyzed so as to show their correlation with other quantities or with structural phenomena, and, moreover, little indication is given of the dependence that can be placed on the data, for the variability due to technique in preparation and measurement is neglected. The present article is written to indicate how these defects may be made good, to show what kind of results may be obtained, and to demonstrate, if possible, how large the collection ought to be to give satisfactory results. The number of prepared specimens of ferret pronuclei in existence is very limited, and in such a case the determination of the number of specimens desired is one of the most fundamental investigations.

For historical information on nuclear and cytoplasmic measurements, reference may be made to the work mentioned in a recent article by the author (Mainland, '31 a), and also to the articles by Shull ('22), Erdmann ('11), and Koehler ('12). Much of this kind of work has been done on invertebrate material, some of it being on embryonic stages, but even in such cases the stages are mostly postpronuclear.

MATERIAL AND METHODS

The observations discussed here were made at the same time as the structure of the ferret pronuclei was studied (Mainland, '30), and the series of specimens was the same in both cases. Measurements were made on the pronuclei of fifty-two ova, some fixed in Zenker's fluid, some in Perenyi's, and a few in Mann's fluid, the staining methods being Heidenhain's iron-haematoxylin and Mayer's haemalum. The ova were in paraffin sections cut at 10 μ .

Details of the method of measurement and calculation have been given in the paper mentioned above (Mainland, '31 a), in which tests were recorded showing the variability due to experimental error. It is sufficient here to state that the volumes and areas were calculated from three dimensions of each pronucleus: 1) The long axis in the largest section of each pronucleus; 2) the transverse axis, i.e., at right angles to 1, the accuracy of the position of this second axis being insured by rotation of the microscope stage from one fixed point to another 90° distant; 3) depth, determined by the graduated fine adjustment of the microscope. In the article referred to, the use of depth measurement is discussed and justified and its importance emphasized.

The volumes were calculated from the formula for the volume of an ellipsoid: $\frac{4}{3}\pi\frac{a}{2}\frac{b}{2}\frac{c}{2}$, where a , b , c are the axes. The areas of the surfaces of the pronuclei were calculated from the formula $\pi(ab + bc + ca)$, kindly computed for the purpose by Prof. N. R. Wilson, of the University of Manitoba.

RESULTS

The accompanying table (table 1) shows the result of the calculation of volume and area. All the results obtained are given in the table, but in subsequent discussions only those pronuclei are considered that occurred in pairs. Where only one pronucleus could be satisfactorily measured, the results were not taken into account.

*Comparison of volumes and areas**Central pronuclei (35 pairs)*

Mean volume of larger pronuclei: 4628.2 cu. μ
 Standard deviation of series: ± 1584.0
 Coefficient of variation: 34.2 per cent
 Probable error of coefficient of variation: ± 3.06
 Mean area of larger pronuclei: 1332.6 sq. μ
 Standard deviation of series: ± 307.4
 Coefficient of variation: 23.1 per cent
 Probable error of coefficient of variation: ± 1.96
 Difference between coefficients of variation of volume and area: 11.1
 Probable error of difference: ± 3.63

The difference is therefore significant, for it is more than three times its probable error.

Mean volume of smaller pronuclei: 3516.2 cu. μ
 Standard deviation of series: ± 1371.0
 Coefficient of variation: 39.0 per cent
 Probable error of coefficient of variation: ± 3.58
 Mean area of smaller pronuclei: 1111.1 sq. μ
 Standard deviation of series: ± 292.8
 Coefficient of variation: 26.4 per cent
 Probable error of coefficient of variation: ± 2.27
 Difference between coefficients of variation of volume and area: 12.6
 Probable error of difference: ± 4.24

The difference here is almost significant.

The reason for the enlargement of the pronuclei after insemination is not known in detail. It is not clear whether the effect, and perhaps the main object, is the establishment of a certain volume, or the establishment of a certain area of surface. The results just given suggest, at first sight, an answer to the question. The differences of size of the ova probably influence the differences in size of the pronuclei, but in these data the ova were the same (thirty-five specimens) and the comparison was made between the differences of volume on the one hand and the differences of area on the other. The areas are much less variable than the volumes, and from this it might be supposed that a certain area rather than a certain volume was the aim or effect, at least, of the enlargement. When reference is made, however, to the measurements of a single pair of test pronuclei (Mainland,

TABLE 1
Volumes and areas of pronuclei

NO. OF OVUM	PRONUCLEUS	VOLUME (μ^3)	RATIO OF VOLUMES ($\frac{\text{GREATER}}{\text{LESSER}}$)	AREA (μ^2)	RATIO OF AREAS ($\frac{\text{GREATER}}{\text{LESSER}}$)
A. Pronuclei central in ovum					
2	A	4171.0	1.55	1253.0	1.33
	B	2697.0		942.0	
3	A	2908.0	1.45	987.4	1.28
	B	4206.0		1261.0	
4	A	5399.0	1.93	1488.0	1.55
	B	2794.0		965.2	
5	A	5746.0	1.19	1553.0	1.12
	B	4828.0		1386.0	
6	A	3782.0	1.20	1174.0	1.14
	B	4546.0		1338.0	
14	A	4019.0	2.04	1233.0	1.60
	B	1975.0		768.6	
16	A	2291.0	1.18	837.1	1.11
	B	1936.0		757.1	
17	A	5161.0	1.47	1458.0	1.28
	B	3513.0		1138.0	
18	A	5492.0	1.25	1571.0	1.10
	B	4393.0		1424.0	
19	A	2977.0	1.24	1012.0	1.15
	B	3689.0		1168.0	
20	A	5451.0	1.02	1509.0	1.03
	B	5586.0		1550.0	
22	A	6042.0	1.24	1632.0	1.16
	B	4874.0		1410.0	
23	A	7837.0	1.78	1909.0	1.46
	B	4393.0		1304.0	
47	A	5439.0	1.74	1504.0	1.44
	B	3123.0		1041.0	
55	A	3387.0	1.35	1093.0	1.21
	B	2509.0		901.4	
57	A	2609.0	1.23	918.4	1.15
	B	3217.0		1055.0	
58	A	4111.0	1.37	1242.0	1.23
	B	3010.0		1013.0	
59	A	2764.0	1.56	953.2	1.34
	B	4290.0		1277.0	
60	A	4568.0	1.31	1372.0	1.20
	B	3481.0		1142.0	
62	A	2174.0	1.13	784.1	1.13
	B	2445.0		893.4	
63	A	1912.0	1.14	683.4	1.02
	B	1680.0		699.3	
64	A	2266.0	1.63	848.5	1.22
	B	1579.0		697.5	
85	A	4597.0	1.14	1340.0	1.09
	B	5227.0		1458.0	
86	A	5532.0	1.23	1515.0	1.14
	B	4501.0		1324.0	
87	A	2038.0	1.27	779.9	1.18
	B	2580.0		910.4	
88	A	4506.0	1.37	1326.0	1.23
	B	6177.0		1636.0	
89	A	5195.0	1.56	1470.0	1.34
	B	3336.0		1101.0	

TABLE 1—(Continued)

NO. OF OVUM	PRONUCLEUS	VOLUME (μ^3)	RATIO OF VOLUMES ($\frac{\text{GREATER}}{\text{LESSER}}$)	AREA (μ^2)	RATIO OF AREAS ($\frac{\text{GREATER}}{\text{LESSER}}$)
92	A	2966.0	1.31	1001.0	1.19
	B	3870.0		1194.0	
93	A	1782.0	1.88	724.3	1.50
	B	3345.0		1087.0	
94	A	2086.0	1.12	761.1	1.26
	B	2336.0		960.6	
95	A	3826.0	1.08	1158.0	1.03
	B	3542.0		1128.0	
96	A	5191.0	1.14	1486.0	1.07
	B	5896.0		1586.0	
98	A	6790.0	1.08	1733.0	1.04
	B	6287.0		1661.0	
99	A	7005.0	1.21	1698.0	1.09
	B	5772.0		1561.0	
100	A	7012.0	1.20	1782.0	1.12
	B	8389.0		2001.0	
45	A	2827.0	1.09	971.0	1.06
	B	2894.0	1.12	985.4	1.08
	C (subcentral)	2584.0		912.2	
B. Pronuclei subcentral in ovum					
8	A	205.3	1.20	168.3	1.13
	B	244.9		189.7	
44	A	3315.0	1.32	1093.0	1.22
	B	2513.0		893.2	
C. Pronuclei subcentral and peripheral					
43	A (peripheral)	479.2	1.31	301.6	1.18
	B (subcentral)	626.2		353.2	
D. Pronuclei central and peripheral					
15	A (central)	2310.0	1.62	849.1	1.38
	B (peripheral)	1422.0		613.7	
E. Pronuclei peripheral					
7	A	358.4	11.86	244.9	5.07
	B	30.20		48.3	
9	A	22.8		39.2	
10	A	24.11	31.32	40.5	10.02
	B	754.9		405.2	
11	A	24.02	1.68	40.4	1.35
	B	14.3		30.0	
28	A	313.9		223.5	
29	A	139.8	1.10	130.5	1.07
	B	126.3		122.1	
30	A	551.0	3.26	325.4	2.15
	B	168.8		151.6	
36	A	150.4		138.7	
41	A	117.5		118.5	
48	A	60.0		77.2	
91	A	356.2	3.88	246.8	2.43
	B	1383.0		600.8	
97	A	600.8	1.08	351.1	1.07
	B	556.2		327.4	

'31 a), it will be seen that this explanation does not hold. In these tests the coefficient of variation of volume in twenty-five measurements of the smaller pronucleus was 6.5 ± 0.623 per cent and the coefficient of variation of area calculated from the same measurements was 4.2 ± 0.396 per cent. The difference was 2.3 and its probable error was ± 0.72 . The difference is therefore significant. When the measurements of volume and area of the other pronucleus were compared with each other, there was found to be a difference in the variation that was almost significant, as in the case of the smaller pronuclei in the present series of thirty-five pairs. Although the variability was much greater in the present series than in the series of measurements made on a single pair, yet the area measurements varied less in both cases, and one can account in both cases for this by the methods of measurement and calculation, for it is obvious that such an explanation accounts for the difference when the same pronucleus was used for the calculations. The object of the enlargement of the pronuclei cannot be shown to be the establishment of a definite area for metabolic interchange between pronuclei and cytoplasm.

Comparison between central and peripheral pronuclei in respect of size

The numbers of observations in this series are unfortunately small (seven pairs), but the results are nevertheless of some interest.

Mean volume of larger pronuclei: 544.6 cu. μ
Standard deviation of series: ± 417.5
Coefficient of variation: 76.7 per cent
Mean volume of smaller pronuclei: 182.3 cu. μ
Standard deviation of series: ± 188.9
Coefficient of variation: 103.6 per cent
Mean area of larger pronuclei: 299.7 sq. μ
Standard deviation of series: ± 170.6
Coefficient of variation: 56.9 per cent
Mean area of smaller pronuclei: 138.1 sq. μ
Standard deviation of series: ± 104.9
Coefficient of variation: 76.1 per cent

In each case the variation between peripheral pronuclei is greater than the corresponding variation between central pronuclei. In spite of the smallness of the numbers, it is evident that when the pronuclei are peripheral they undergo greater changes of size than after they have become established at the center of the ovum. This view is confirmed by comparison of specimens such as nos. 8 and 44 (table 1, B). In both of these the pronuclei are subcentral and yet there are very great differences in pronuclear size.

Combined areas of central pronuclei

Since the areas were less variable than the volumes, it was decided to consider only the former as a measure of size. When the areas of the two pronuclei are added together, it may be assumed that to some extent the sum is an index of development—that the greater sums will be found at the later stages—but, on the other hand, this might be counterbalanced by a secondary diminution in size, perhaps preparatory to segmentation. If there is a natural tendency for the central pronuclei to reach a maximum area and to maintain it, the frequency-distribution curve of combined pronuclear areas should be skewed to the right, that is, most of the specimens should be found to possess areas greater than the average. The thirty-five pairs of central pronuclei here recorded are insufficient to prove this conclusively, but the figures suggest that the hypothesis is correct.

Combined areas of pairs of central pronuclei

<i>Sq. μ</i>	
1001-1500	1
1501-2000	8
2001-2500	9
2501-3000	10
3001-3500	7
	—
	35

The only external measure of developmental stages available for these ova was the length of the period between insemination and the killing of the animal, and the limitations

of this measure have been shown elsewhere (Robinson, '18; Mainland, '30). The mean combined area of central pronuclei at the different postinseminal periods is shown below:

<i>Hours after insemination</i>	<i>Sq.μ</i>	
41 $\frac{1}{2}$	1797.9	(2 observations)
47 $\frac{1}{2}$	2664.6	(9 observations)
51 $\frac{3}{4}$	2725.5	(2 observations)
64 $\frac{1}{4}$	2549.8	(12 observations)
76 $\frac{1}{2}$	2545.0	(1 observation)
116 $\frac{1}{2}$	2097.3	(4 observations)

There is no suggestion of regular changes in the means as the time advances. The number of observations is small and the variation in each time class was found to be great. The ova at forty-seven and one-half hours and at sixty-four and one-fourth hours are most numerous. The difference in the means between these is 114.8 sq.μ, but the probable errors are ± 87.11 and ± 110.79 , respectively, and therefore the difference is not significant. Even if time, as here measured, has some influence, there must be other factors responsible for the great amount of variation in size, apart from the time and apart also from errors of measurement, for the errors of measurement (Mainland, '31) do not produce such great variation as that found here (see above, p. 108). Other factors responsible for the variation might be the size of the ovum (upon which investigations are at present being carried out), temporary metabolic changes, and differences in preparation, e.g., of fixative. The influence of fixatives was studied as follows:

Mean combined area of pairs of central pronuclei

<i>Fixative</i>	<i>Area</i>	<i>Observations</i>
Perenyi	2227.3	9
Zenker	2646.8	23
Mann	1535.4	3

Since the numbers of observations were small, Fisher's *t* test was applied (Fisher, '30, p. 107) in the comparison between Zenker and Perenyi specimens, and the result ($t = 0.65$; $n = 30$) showed that the difference in fixative did not account for size differences.

Ratio of greater area to smaller

The ratio is an expression of the relative areas of the two pronuclei. The greater the difference in size, the greater the ratio. Inspection of table 1 suggests that the ratio is greater in the ova with peripheral pronuclei than in those with central pronuclei. The results were tested as follows:

Mean ratio of areas for thirty-five pairs of central pronuclei: 1.210

Probable error of mean: ± 0.0169

Standard deviation of series: ± 0.149

Coefficient of variation of series: 12.3 per cent

Mean ratio for seven pairs of peripheral pronuclei: 3.308

Probable error of mean: ± 0.77

Standard deviation of series: ± 3.03

Coefficient of variation of series: 91 per cent

Difference between means: 2.098 ± 0.77 ; not significant

The difference between the means of the central and peripheral pronuclei is not significant, owing to the smallness of the numbers of peripheral pronuclei and to the great variation in size of these, but the difference between the ratios is made clear by taking the extreme possible ratio for the central pronuclei, which can be estimated from the standard deviation of the series (0.149). Three times this (i.e., 0.45) added to the mean gives the highest possible ratio to be expected in the series, i.e., 1.66. Now five out of the seven ratios of peripheral pronuclei are above this limit (one being 5.075 and another 10.020). Thus the peripheral pronuclei tend to have very much higher ratios than the central pronuclei; that is, the members of a pair of peripheral are very much more apt to differ widely in size than are the members of a pair of central pronuclei.

Several questions might be raised with regard to the ratio of areas in the case of the central pronuclei. It might be asked whether, in an ovum that was going to give rise to a female, the ratio was different from that in an ovum destined to become a male, that is, whether the chromatin constitution of the pronucleus influenced the ratio. Table 2 shows the ratios arranged according to the animal from which the ova came. It will be seen that the ratios do not tend to group

themselves in two classes, although certain of the ova must be males and certain must be females. If there is any influence of chromatin constitution, its effect is obliterated by variation due to other causes.

The totals given in the penultimate row of table 2 show that the frequency-distribution curve would be skewed to the left. The numbers are small, but there seems to be a tendency for the majority of the central pronuclei to be nearly equal in area rather than very different. It may next be asked whether the central pronuclei do not tend more and more

TABLE 2

Ratios between areas of central pronuclei—numbers of ova arranged according to ratios

DESIGNATION OF ANIMAL	RATIOS OF AREAS						TOTAL
	1.01-1.10	1.11-1.20	1.21-1.30	1.31-1.40	1.41-1.50	1.51-1.60	
26.7	5	3	2	1	1		12
G 9	1	2	1				4
DA 5 R					1		1
GB 4	3	3			1	1	8
DA 38		2					2
DA 33			1	1		1	3
FZ 1.1		2	2	1			5
Total	9	12	6	3	3	2	35
Mean combined area of pairs of pronuclei (μ^2)	2760.2	2411.6	2121.3	2332.1	2523.1	2227.4	

to arrive at a ratio near unity as their development proceeds. This was tested by taking a mean combined area for each class of ratio (table 2). There appeared to be some tendency for the greater combined area to be associated with the smaller ratio. A straight-line regression equation was calculated by Fisher's method (Fisher, '30), to show whether the mean combined areas could be arranged in a straight line descending as the ratios increased. The variations in the individual items were so great, however, that the *t* test showed that the slope of the straight line was not significantly different from the horizontal, that is, the areas remained, so far as could be shown, constant regardless of the ratios.

Relative sizes of central pronuclei

The size difference between the two members of a pair of central pronuclei is chiefly of interest because of the sexual difference between the two. It becomes desirable to differentiate in as many ways as possible the two pronuclei, and if relative size can be taken as an index of sexual difference, it may be of great importance in ova where it is not possible to distinguish the male pronucleus from the female by the obvious characteristic—the possession of a sperm tail.

There was one ovum (no. 88) in this series that contained an undoubted sperm tail in its cytoplasm (Mainland, '30), and the pronucleus near which it was found was the smaller (table 1). Unless the pronuclei have been displaced, this appears to show that the male pronucleus is the smaller. In the guinea-pig the male pronucleus is stated by Lams ('13) to be the larger, and the figures of guinea-pig ova shown by O. van der Stricht ('23) reveal the fact that the numbers upon which this statement was based are quite adequate to exclude pathological or freak specimens. Hill and Tribe ('24) describe two ova of the cat in each of which there was a supernumerary pronucleus. In these specimens there was a large and a small central pronucleus, while the extra pronucleus was comparable in size to the larger central pronucleus. The authors concluded that in the cat, as in the guinea-pig, the larger pronucleus was the male. The argument has a certain weakness, perhaps, because it is based on abnormal specimens. One specimen of the series of ferret ova was comparable with these of Hill and Tribe (no. 45, table 1 at end of section A). In this the two central pronuclei were of nearly equal size. The extra pronucleus (pronucleus C), presumably the male, was smaller than either of them. In the ferret, therefore, there are two specimens, both in agreement, which suggest that the smaller pronucleus is the male.

In the description of the pronuclei referred to above (Mainland, '30) there was a discussion of various structural differences between the two pronuclei, e.g., the presence in certain

of the central pronuclei of eosinophilic or pale globules, differences in shape and size of the chromatin particles, differences in the reticulum of the pronuclei. The two pronuclei of one ovum differed so seldom from each other in these respects that it was impossible to distinguish the male from the female or the larger from the smaller by any of these structural peculiarities. The peculiarities were due to factors influencing both pronuclei, in some cases technique, in others the developmental stage.

Relation of central pronuclei and polar bodies

Table 3 shows the arrangement of pronuclei with reference to polar bodies. The chi-square test showed that the actual arrangement differed from the theoretical random arrange-

TABLE 3
Arrangement of central pronuclei with reference to polar bodies

NUMBER OF OVA	LARGER PRONUCLEUS NEARER	SMALLER PRONUCLEUS NEARER	PRONUCLEI EQUIDISTANT	TOTAL
Actual	5	16	3	24
Theoretical (random distribution)	8	8	8	24

$$\chi^2 = 12.25; n = 2; P \text{ is less than } 0.01.$$

ment, and even when the three ova with equidistant pronuclei were omitted, the chi-square test result was still significant. The conclusion is that there is a definite tendency for the smaller of the two central pronuclei to be nearer the polar body.

In the guinea-pig (Lams, '13) the larger pronucleus is nearer the point of emission of the polar bodies, and, as mentioned above, in that animal the larger pronucleus is probably the male. It has already been shown that the evidence points to the male pronucleus as the smaller in the ferret, and it has now been shown that the smaller pronucleus is nearer the polar bodies. This appears to confirm the previous suggestion that the male is the smaller pronucleus, for the phenomena suggest the following statement: In some

animals the male, and in others the female, is the smaller of the central pronuclei, but in either case the male pronucleus tends to be nearer the point at which the polar bodies are found.

The significance of this relationship to the polar bodies requires a little further discussion. It has been shown (Mainland, '31 b) that the ferret ovum has a polar distribution of its cytoplasm, i.e., there is a granular pole and a pole at which more deutoplasm or lipoid material is assembled. It has been further shown that polar bodies tend to be located nearer the granular pole rather than the deutoplasmic pole. The polar bodies, therefore, although severed quickly from the ovum, tend to form a landmark with reference to the granular pole, which is therefore the 'animal' pole of the ovum. One may therefore suggest that the pronuclear arrangement is such that the smaller tends to be turned toward the 'animal' pole. A direct test was made of this suggestion, and the result was at first sight in disagreement with it, for out of seventeen ova, eight showed the larger central pronucleus near the granular zone, seven showed the smaller pronucleus nearer, and in two ova the pronuclei were equidistant. The arrangement therefore appeared a random one. It was shown, however, by the chi-square test that the data just recorded could also agree with those obtained above for the distribution of the pronuclei with reference to the polar bodies. The disagreement, therefore, was only apparent.

Absolute size of central pronuclei

The size (area of surface) of the central pronucleus may be taken as an index of development, for it is known in general that the pronucleus enlarges in the course of its development, and it is in particular indicated here that the pronucleus worked up toward a maximum and did not thereafter change greatly in size (see section on combined areas of pronuclei with reference to their frequency distribution). It might be expected, therefore, that some of the features of

pronuclear structure previously described (Mainland, '30) ought to be correlated with the development of the pronucleus in this way.

1. Sizes of particles of chromatin

<i>Number of pronuclei</i>	<i>Size of particle most notable in pronucleus</i>	<i>Mean area of pronucleus (μ^2)</i>
4	Small	1363.5
6	Medium	1089.4
10	Large	1341.8
32	Various	1273.9

Without much statistical analysis, it was clear that there was no association between pronuclear area and size of chromatin particle.

2. Fineness of reticulum

<i>Number of pronuclei</i>	<i>Reticulum</i>	<i>Mean area of pronucleus (μ^2)</i>
17	Fine	1256.2
9	Medium	1298.6
16	Coarse	1290.5

Again, inspection of the variation among the data from which these averages have been obtained showed, without the calculation of probable errors, that there was no significant relationship demonstrable between the reticulum and the size of the pronuclei.

3. Presence of eosinophilic, pale, or colorless globules

<i>Number of pronuclei</i>	<i>Globules</i>	<i>Mean area of pronucleus (μ^2)</i>
18	None pale	1388.6 ± 12.53
32	Some pale	1426.7 ± 39.56

Here also there was no significant difference. It is easily observed that the very small peripheral pronucleus is basophilic, but the data just recorded show among the central pronuclei no association between size (area) and structure. If there is any association, it is too small to show through the great variation present in the pronuclear sizes.

The facts thus elicited by measurement of the pronuclei appear to be largely negative, and one must endeavor to ascertain what positive contribution they have made to our knowledge of pronuclear development. It has been already

shown (Mainland, '30) that, even in a collection of forty-three ferret ova, obtained some early and some late in the stage of central pronuclei, it is impossible to demonstrate a definite sequence of pronuclear transformations. It is now shown that the sizes of the pronuclei at this stage are very variable. They are much more variable, indeed, than was suspected by the author when making the observations. This fact in itself affords justification for the investigation. Not only are the sizes very variable, but are, so far as can be shown, not associated with structural differences. This fact is bound to modify our conception of pronuclear development. One may conceive of the pronuclei enlarging and coincidentally proceeding through a series of structural changes; but the evidence so far obtained does not support this conception.

It is quite probable that the negative character of the results obtained is largely due to the great variability of pronuclear size. When such variability has been demonstrated, the further object of the investigator is to show how large the series of specimens should be to compensate for the variability. A test of this kind was made on the series quoted above in which there was a comparison of areas of pronuclei with regard to the presence or absence of eosinophilic globules. It was assumed that the means and the variability remained the same as the numbers of observations increased. For the difference to be significant, the probable error would need to be at most $38/3$, say 12. x was assumed to be the number of times the observations, i.e., number of specimens, required to be increased. The probable errors would then be $\frac{1}{\sqrt{x}}$ of the present ones. It was then shown by a solution of a simple equation that the value of x would require to be about 12. Therefore the observations would have to be increased nearly twelve times to make the difference between the means significant. On the other hand, of course, as the observations increased the variability might also increase, or the means might become less different.

Further investigation is therefore indicated. This investigation may proceed in two directions, both by greatly increasing the numbers of specimens and by determining the relation of the ovum size to the pronuclear size.

SUMMARY

Measurements have been made of the pronuclei in the paraffin sections of fifty-two ferret ova. The volumes and areas have been calculated by the use of the formulae for an ellipsoid, and the results have been analyzed statistically. There is great variation between the areas of the central pronuclei of thirty-five different ova. There is even greater variation between the corresponding volumes, but this does not prove that a definite area of external surface is the object or result of pronuclear enlargement, for in a previous investigation (Mainland, '31a) a series of measurements of the same pair of pronuclei was made and the resulting volumes varied more than the corresponding areas.

The peripheral pronuclei were few (seven pairs), but these pronuclei vary from ovum to ovum much more than the central pronuclei.

Owing to the smaller variation in areas, these were used, instead of the volumes, in the subsequent investigations. The two areas of each pair of central pronuclei were added together and arranged in a frequency-distribution series. Although the series is too small to give definite proof, it shows that as the size increases the numbers increase and then more rapidly decline. This suggests that the pronuclei enlarge up to a maximum and do not subsequently diminish.

There is no indication that the size of the central pronuclei increases as the time after insemination increases.

The difference in fixative has no apparent influence on pronuclear size.

The ratio between the areas of the two members of each pair of pronuclei was found by dividing the area of the greater by the area of the smaller.

There is a tendency for the central pronuclei to be nearly the same in area, rather than very different, for there is no ratio above 1.60.

The pronuclear ratio is not demonstrably influenced by the fact that some of the ova were destined to give rise to males and others to females.

It appears as if the pronuclei with the greater combined area (i.e., presumably, those more advanced in development) tend to have a ratio nearer unity than the rest, but this cannot be conclusively proved from this material.

The pairs of peripheral pronuclei tend to have much greater ratios (e.g., over 10.0) than the central pronuclei; that is, the two peripheral pronuclei differ more from each other in size than do the central pronuclei.

One ovum containing a sperm tail and one ovum with a supernumerary pronucleus are in agreement in suggesting that the male pronucleus is in the ferret the smaller.

The smaller of the central pronuclei is in a significant majority of cases nearer the polar bodies, that is, nearer the 'animal pole' of the ovum, than is the larger pronucleus.

There is no significant association between the size (area) of a pronucleus and, 1) the sizes of its chromatin particles, 2) the fineness of its reticulum, 3) the presence in it of eosinophilic, pale, or colorless globules.

A test has been carried out with the data on the eosinophilic or pale globules, to show that, if the variability of the data and the difference between the average pronuclear areas remained the same, the number of observations would have to be multiplied by twelve to render this difference significant.

Further investigations should involve increasing the number of specimens and studying the relationship of ovum size to pronuclear size.¹

¹ Results obtained while this article was in press indicate that the great variations in pronuclear size discussed here are independent of variations in ovum size.

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I wish to express again my indebtedness to Prof. Arthur Robinson for the loan of the ova used in this investigation, to the trustees of the Moray Fund of Edinburgh University for financial assistance in the preparation of certain of them, and to my wife for assistance in carrying out most of the calculations.

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SUPRAVITAL STUDIES ON THE ERYTHROCYTE
OF THE CATFISH (AMEIURUS NEBULOSUS,
LESUEUR), WITH SPECIAL REFERENCE
TO THE NITTIS STIGMA

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TEN FIGURES

This brief paper on the erythrocyte of the catfish is prompted by the rather categorical pronouncement of Nittis ('30) that the stigma as described by him is a specific structure of the erythrocyte not to be confused with other granules, globules, and vacuoles which react with vital dyes, and is apparently common to all circulating, mature, nucleated red blood cells. This generalization is apparently based on a study of the blood of four different animals, frog, pigeon, horned toad, and snake; with the exception of the horned toad, the exact species utilized are not definitely indicated. If the blood cells of the different species of frogs and snakes are as variable in their response to vital dyes as are those of the urodeles (Dawson and Charipper, '29), the exact identification of the specimens from which blood was obtained is a matter of some importance and should not have been omitted from the report. Nittis also notes that the author (Dawson, '28, '30) failed to recognize the stigmata as discrete units in the erythrocytes of *Necturus*. It must be admitted that even the method of applying brilliant cresyl blue as described by Nittis has, in my hands, failed to reveal any such structure in these cells. However, in the erythrocytes of the catfish certain structures have been observed which possess some of the features which Nittis regards as specific for the stigmata, while on the other hand a careful study shows that they

undoubtedly belong to the category of granules, globules, and vacuoles which have been described by so many investigators as cytoplasmic inclusions in many nucleated erythrocytes.

The stigma of the erythrocyte, as described by Nittis, was demonstrated by brilliant cresyl blue applied supravitaly as a saturated solution in 0.85 per cent sodium chloride. It appears as a single, sharply outlined body either on the erythrocytic membrane or very near the surface of the cell. As a rule, it is found on the upper surface and is located eccentrically. The stain was taken up by the constituents of the cell in the following order: the stigma was the first to take the stain, then the nucleus; this was followed by the reticulum, and, lastly, by the appearance of another stigma. This second stigma was generally smaller than the first and lay on the opposite surface of the cell—as a rule, the lower. Its size was about one-half or three-quarters of that of the first stigma. The two stigmata never appeared to be situated on the same plane, and when one was visible the other was out of focus. The distance between the two was estimated, by focusing the microscope, to be approximately equal to the thickness of the erythrocyte. When higher concentrations of the dye were used, there also appeared a thread-like line of particles that extended from underneath the first stigma to the nucleus, and even when the reticulum was stained the stigma was much more deeply colored than any of the granules observed within the cytoplasm either in connection with, or independent of, the reticulum.

In contrast to the other granules which may appear in the cytoplasm, this body never exhibited brownian motion. Furthermore, it could not be colored with Wright's stain, whether applied routinely to a dry film or preceded by supravital staining with brilliant cresyl blue.

Nittis makes several suggestions regarding the nature of the stigma. It might indicate a hollow cavity of the surface membrane in which the stain accumulates, or it might possibly be the point of separation of the erythrocyte from the mother or sister cell. He appeared to feel, however, that the

interpretation which best satisfied the facts would be to regard the stigma as a part of a microcanalicular or minute circulatory system extending from the large stigma (stoma) on the surface of the cell to the nucleus, then through the meshes of the reticulum to end on the opposite surface of the membrane in the second or smaller stigma (a sort of cytopye).

The staining reactions of the stigma do not present any difficulties to an investigator familiar with the behavior of the intracellular inclusions of the nucleated erythrocyte when stained supravitaly. The stigma behaves in a manner very similar to the primary or pre-existing bodies of the erythrocyte of *Necturus* and many other vertebrates (Dawson, '28, '30; Dawson and Charipper, '29; Dornesco and Steopoe, '30 a, '30 b) which is to be contrasted with the delayed reaction of the induced bodies which may be independent of, or associated with, reticulation within the cell.

The two most striking features of the large stigma, as described by Nittis, are first, that it never exhibited brownian motion and, secondly, that as a rule, it was situated on the upper surface of the cell. The absence of any brownian activity apparently led Nittis to conclude that the stigma was probably situated on the membrane of the cell. Its usual location on the upper surface of the cell is difficult to explain. It seems very improbable that such flattened objects as erythrocytes should always come to lie with the same side uppermost, since the stigma could not possibly be large enough to influence the manner in which the erythrocytes settle when suspended in serum in a moist preparation. This constancy of the location of the stigma on cells which presumably come to rest on one side or the other at random implies either that the stigma is an artefact resulting from the manner in which the cells are manipulated during the supravital staining, or that it is a pre-existent body free to change its location or orientation, under the influence of some force such as gravity, after the blood cells have come to rest. Nittis' additional observation on the secondary appearance

of a smaller stigma on the lower surface of the cell throws no light on this problem of constancy in location and orientation.

DESCRIPTION OF MATERIAL

The blood used in this study was taken from catfish varying from 6 to 8 inches in length, and was aspirated directly from a small blood vessel on the inner surface of the operculum. Blood so taken was placed directly on the slide and was accordingly free from contact with either tissue juice or moisture from the surface of the animal. These precautions are essential in securing normal preparations of blood from aquatic vertebrates. Both brilliant cresyl blue and neutral red were applied supravitaly, either as dry films of stain on the slide or by adding saturated solutions of these dyes in 0.85 per cent sodium chloride directly to the blood, usually by allowing the solution to flow under the cover-slip. In many instances Janus green B was added to the neutral red, especially in the dry film method. In all cases the preparations were sealed with warm vaselin to protect them from evaporation during the prolonged study which is frequently necessary. The method of supravital staining of blood cells by the previous filming of the slide with an alcoholic solution of the dye has certain obvious advantages over the method of introducing small amounts of a saturated solution of dye (in physiological saline solution) directly into the blood. With the first method a film of blood cells more or less uniform in thickness is directly exposed to a uniform film of dry stain, whereas in the second method a staining gradient is set up which itself is not constant, but is continuously changing as diffusion of the dye takes place. Cells in immediate contact with the dye are accordingly exposed to a maximum concentration, while those farther away are exposed to a lower and continuously increasing concentration. Furthermore, with the first method it is possible to determine empirically the optimum concentration for the particular blood for the question under investigation. In the supravital studies

on the erythrocytes of *Necturus* it was possible to apply neutral red in such limited quantities that only the pre-existing bodies reacted with the dye even on exposure for forty-eight hours or longer, while higher concentrations quickly induced the appearance of secondary granules and even produced typical patterns of reticulation.

The same precautions were taken in staining the erythrocytes of the catfish. It was found possible to apply both neutral red and brilliant cresyl blue, by the dry-dye-film method, in such limited concentrations that little or no staining of secondary granules, nucleus, or reticulum was obtained in preparations kept for forty-eight hours at room temperature. When saturated solutions of the dyes in normal saline were added to fresh blood, the picture presented was much more variable, many cells showing stained nuclei, secondary or induced granules, and elaborate patterns of reticulation.

In preparations stained with a so-called optimum or limiting concentration of either dye, the erythrocytes on immediate examination are seen to contain what appears to be a single, brightly stained dot or granule, situated eccentrically. If Janus green B was added to the neutral red, there later appeared a variable number of relatively large spherical mitochondria which are closely grouped around the nucleus (fig. 1).

Unlike the stigma of *Nittis*, the densely stained granule is not always fixed in position, but exhibits definite brownian movements. As the granule moves it is seen to be accompanied by a much smaller body which lies deeper within the cell. The two granules appear to be joined by a delicate filament which cannot be distinctly seen, since it does not take the stain. In other words, the structure resembles a dumb-bell with terminal balls of unequal size, the larger ball lying uppermost in the cell.

The activity of this structure is not always confined to the short irregular motions characteristic of brownian movement. It also exhibits swaying motions during which the lower granule is seen distinctly at times and at others is

completely obscured by the larger upper one. At irregular intervals this structure is seen to move rather rapidly toward the nucleus and then back toward the margin of the cell (fig. 10). Two or three such back and forth movements may occur before the inclusion settles down again to the simpler brownian activity. Occasionally the structure may start off at a

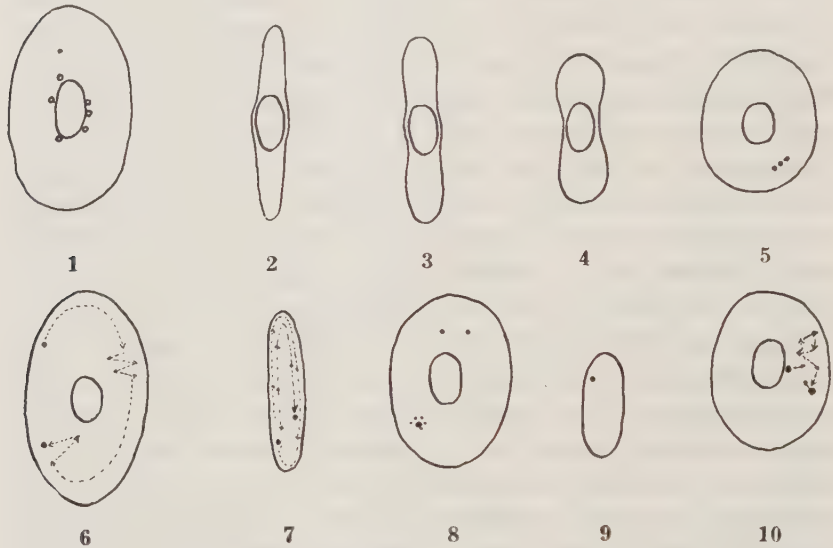


Fig. 1 Normal erythrocyte showing shape and size of the cell, the perinuclear grouping of the chondriosomes, and the small eccentrically placed granule. (Neutral red-Janus green.)

Fig. 2 A normal erythrocyte viewed on edge.

Figs. 3 and 4 Modified erythrocytes viewed on edge, showing the changes in shape which occur in supravital stained preparations after several hours.

Fig. 5 A similar cell, seen in surface view, showing three neutral-red granules of equal size and lying in the same plane. The abnormal number and size of the granules are due to the prolonged exposure to the vital stain.

Figs. 6 and 7 Diagrammatic sketches to show the paths taken by the vitally stained granules through the cytoplasm in the more extensive movements which occasionally occurred. In figure 6 the cell is lying flat on the side, in figure 7 it is on edge.

Fig. 8 A cell containing several neutral-red bodies, the result of induction. The lower cluster represents an extension of the original inclusions, while the two upper bodies are entirely new formations.

Fig. 9 A modified cell seen on end.

Fig. 10 A diagrammatic sketch to show the more localized movements between the nucleus and the cell membranes of the vitally stained granules.

fairly even speed and move for considerable distances around the cell (fig. 6). Sometimes a complete circuit of the cell was made, and in a few instances a second lap was almost completed (fig. 7). The path followed does not appear to be fixed and the dumbbell-like body may at times be near the margin of the cell and at others quite near the nucleus. When close to the surface membrane, the structure often seems to bob along as if tending to come to rest, only to be swept along by an invisible current. During most of these translatory movements only the larger granule is in evidence, but occasional glimpses of the lower granule are caught as the structure sways.

The foregoing description of the appearance, orientation, and movements of the vitally stained body has been limited to observations made while the erythrocytes lay flat on the slide, but in thicker films it was possible to find many cells which had come to rest 'on edge.' In a few instances, judging from the dimensions of the part of the cell seen in sharp focus, it appeared that some cells were even 'on end.' In many such cells the vitally stained granules were visible and appeared to be in close contact with the portion of the surface membrane which was uppermost in the preparation (figs. 7, 9). This implies that the granules, in certain instances at least, are free to move through the cell. The orientation, with the larger granule uppermost, was the same as in the cells lying flat on the slide and the movements exhibited were also the same as previously noted. The rather definite orientation observed in all cases suggests that there is some force determining the position assumed by the conjoined granules. This will be referred to later.

The observations just recorded were made on preparations in which the concentration of the dye was kept so low that little or no induction of secondary granules occurred even in preparations which had stood for forty-eight hours, and this factor apparently is responsible for the ease with which the vitally stained granules can be observed executing the several movements which have been described.

When the preparations are freshly made, the degree of brownian activity is very limited and can barely be detected. Later there is, in many cases, a distinct increase in size, especially of the upper large granule, and the brownian activity becomes much more pronounced. In many cases it appeared that the lower, smaller granule was a secondary or induced one. At least, it only becomes distinctly visible after the preparation has been under observation for some time. The increase in brownian activity is apparently due to a decrease in the viscosity of cell contents, and associated with the decreased viscosity there is also a clearing of the cell, as if either some hemoglobin were lost or the cell had taken up fluid from the surrounding plasma.

Besides the changes in the viscosity and density of the cell contents, the form of the cells also changes. They gradually lose their definite oval outline and become more rounded and when observed on edge are seen to become much shorter and thicker. The unmodified cell when seen in this view is narrow and slightly biconvex, with often a slight bulging in the region of the nucleus (fig. 2). Later the periphery of the cell becomes more rounded and thickened, and when viewed on the edge the cell appears definitely biconcave with the exception of a slight bulge at the level of the nucleus (fig. 3). This thickening and rounding progress gradually until in many cells the surface concavities appear considerably deeper and the nuclear bulges disappear (fig. 4). The disappearance of the nuclear bulges is due to the increase in the thickness of the entire cell, so that the nucleus no longer affects the cell contour. These extensive changes in the form of the erythrocytes make it difficult to determine whether the clearing of the cell is due to the loss of respiratory pigment or to the intake of fluid from the plasma medium, since changes in volume of the cell are largely obscured by the changes in its form (fig. 5).

Movements of the conjoined granules, of the second and third type, i.e., rather rapid migrations from the nucleus to the cell membrane and the more extensive movements around

the cell, are doubtlessly brought about by the changes just described. The loss of density of the cell contents, whether through the loss of hemoglobin or through dilution of the concentration of hemoglobin by the absorption of fluid, could possibly cause currents within the cell. The extensive changes in the form of the cell could also conceivably affect the cell contents in the same way, especially if they occurred suddenly or in restricted areas.

When higher concentrations of dyes (neutral red and brilliant cresyl blue), such as saturated solutions in normal saline, are applied directly to the erythrocyte, it seems possible that a more or less irreversible coagulation of the cell contents is brought about and the opportunity for a display of the active brownian movements and the more extensive movements about the cell is not presented. This apparently is the case in a great many instances, since the nucleus usually stains and definite patterns of reticulation develop within the cytoplasm. That these changes at least are irreversible is readily seen in stale preparations in which the erythrocytes are completely laked. In such cells the stained nucleus, granules, and reticulum stand out prominently in an otherwise clear cell. The high concentration of dye used by Nittis may therefore explain in part why the granules observed by him were never seen to exhibit movement of any kind, since they are firmly fixed by the filaments of the reticulum.

The more or less definite orientation of the conjoined granules with the larger granule uppermost and apparently in close contact with the cell membrane suggested that these structures might be influenced by gravity. The suggestion that density is a determining factor is further strengthened by the behavior of the granules in certain cells. In several instances, probably due to the influence of the dye, the smaller granule eventually attained a size equal to its partner, and in these cells the two granules were not oriented at right angles to the upper surface membrane, but lay in the same plane close to the surface of the cell.

The foregoing observations on the orientation of the granules were made with the stage of the microscope in a horizontal position. In order to test whether gravity was exerting any influence, preparations were also studied with the stage in the vertical position. Although such preparations were kept under observation for as long as six hours, no change in the position or orientation of the granules could be recognized. In some cases the stage was also rotated at frequent intervals while in the vertical position, but this also failed to cause any change in the behavior of the stained bodies. Accordingly, it is concluded that density is not the factor determining the orientation and position of the granules within the erythrocytes. No further attempts were made to account for the phenomenon described, since the present communication is primarily concerned with the morphological relationships of the so-called stigmata described by Nittis.

DISCUSSION AND SUMMARY

In this study of the erythrocytes of the catfish the observations of Nittis on the form, orientation, and staining reactions of the structure under discussion, the stigma, are confirmed. As Nittis points out, the so-called stigmata take the stain before any other stainable element and can be readily demonstrated separately. His further statement that their periphery is always sharply outlined, in contrast to other granules observed within the cytoplasm in connection with or independent of the reticulum, is subject to some modification. The appearance of the induced or secondary granules depends on the rapidity with which they are caused to form within the cytoplasm. With low concentrations of dye acting over several hours, secondary granules of equal size, density, and clearness of outline may appear, and in some cases it is impossible to distinguish between pre-existent and induced granules (fig. 8). In the catfish erythrocytes this is particularly true, as frequently the larger secondary granules possess small companion granules similar to those of the pre-existent type. When higher concentrations of dye are used, the picture closely resembles that described by Nittis.

The primary granules of the erythrocytes of the catfish were not demonstrated by Wright's blood stain, but could be distinguished in unstained fresh preparations after the cells had cleared in the manner already described.

In the catfish the brownian movements of the vitally stained granules in freshly prepared blood are scarcely perceptible, but when the preparations become stale and erythrocytes become clearer and their typically flattened oval form is partially lost, the granules exhibit increased and very obvious brownian movements accompanied at times by either striking vibratory movements between the nucleus and the cell membrane or by extensive migrations through the cell. Such movements show clearly that these bodies are not modifications of the cell membrane, but belong to a category of cytoplasmic inclusions which are readily demonstrated by vital dye and which are common to the nucleated erythrocytes of many animals. Accordingly, Nittis' suggestion that the stigmata are fixed bodies on the cell membrane and his conclusion that they are specific structures common to all circulating, mature, nucleated red blood cells seem untenable. The observations on the catfish suggest that the methods used by Nittis in his study of the erythrocytes of the pigeon, horned toad, frog, and snake are largely responsible for this interpretation. Two criticisms of his method are offered: first, that the high concentrations of dye used tended to produce an irreversible coagulation of the cell contents, and, secondly, that continuous observations were not made over a long-enough period of time.

The difficulty of interpretation is apparently increased when the vitally stainable granules are limited to single or double bodies in each erythrocyte. Their nature is much more obvious when they occur as multiple bodies. The varying position of these granules in the erythrocytes of the urodeles has already been described (Dawson and Charipper, '29), where four types of distribution were recognized: single, unipolar; multiple, clustered, unipolar; multiple, clustered, bipolar; and multiple, scattered or diffuse. Somewhat

similar variations in distribution, though not so striking, also occur in fishes (Dornesco and Steopoe, '30 a, '30 b) and in reptiles (Lewis and Lewis, '26). The bodies described by Nittis for the erythrocytes of the frog, snake, horned toad, and pigeon, and those herein described for the catfish, may be regarded as of the single, unipolar type, although a small accessory granule is usually present.

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THE ARTERIAL COLLATERAL RESPONSE IN THE CAT FOLLOWING LIGATION OF THE ABDOMINAL AORTA

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INTRODUCTION

Numerous studies of the collateral circulation in various parts of the body in man and the lower animals have been made, extending back to ancient times. Ligation of the large arteries, with subsequent preservation of the affected parts through collateral channels, has been successfully performed only in relatively few cases. One of the first ligations of the abdominal aorta in man was performed in 1817 by Sir Astley Cooper and, according to Keen ('00), this was repeated by James (1830), Murray ('34), Monteiro ('40), South ('56), McGuire ('68), Stokes ('69), Watson ('69), and Czerny ('70). All of these cases died, though that of Monteiro's survived for ten days. Brooks ('26) has performed an aortic ligation in which the patient, a man, survived for thirteen weeks, dying of intestinal obstruction due to adhesions. In the discussion of Brooks' article, Matas states that he made a ligation of the abdominal aorta, the patient surviving one year, five months, and nine days, dying of tuberculosis.

Much work has also been done on this subject in animal experimentation, chiefly by Halsted ('09), Guthrie ('12), Carrel ('06), and Reichert ('24). However, in reviewing the literature of this field, it was found that no mention was made of the possible influence of the sex of the subject on the collateral response following aortic ligation. This investigation was undertaken with the purpose of discovering whether there was any difference according to sex in the effective collateral response.

I am indebted to Dr. H. H. Collins, of the Department of Zoölogy, and to Dr. Davenport Hooker, of the Department of Anatomy of the University of Pittsburgh, for many valuable suggestions and criticisms. Also a note of appreciation is due my wife, Bessie Dickerson Apgar, for acting as anesthetist in all the surgical operations.

MATERIALS AND METHODS

A. Materials

The animals used for this investigation consisted of twenty-four adult cats (*Felis domesticus*). Sixteen of these cats provided material for the main problem and consisted of two equal groups, one of males, the other of females. Of the remainder, three exhibited prolonged recovery time and, therefore, could not be included in the main problem; three were used to discover the cause of prolonged recovery time, and two were used to test the cause of the difference in recovery time between males and females following ligation of the abdominal aorta.

All of these cats were presumably from one to two years old, though accurate determination of age was impossible, as they were acquired either by gift or purchase. All of the cats received were kept in the laboratory for a minimum of three days prior to the operation in order to eliminate those that were unfit because of disease. The females were neither pregnant nor lactating at the time of the experiment.

B. Methods

1. *Pre-operative technic.* The ligation of the abdominal aorta is a major operation; it is necessary, therefore, to use strictly aseptic technic.

The cat was anesthetized with ether and the operative field prepared as follows: An area bounded by the ribs, the mammae, the hip joint, and the spine on the left side of the animal was denuded of hair. This was accomplished by clipping as much hair as possible from the area with scissors; and then wetting the area with a saturated solution of sodium

sulphide (Hooker, '30), care being taken not to get the depilatory on any other part of the body. Then the operative field was masked with sterilized cloths and painted with tincture of iodine. Both operator and anesthetist wore masks during the operation.

2. *Operative technic.* The skin incision was begun 3 cm. posterior to the last rib and continued for 7 cm. toward the thigh, on a line parallel to the spinal column and midway between the spinal column and the midventral line. The abdominal muscles were divided by blunt dissection, separating the muscle fibers. The peritoneum was gently peeled away from the inner surface of the body wall. (At times, fat obscured the operative field, but the proper use of retractors retarded its encroachment.) As soon as the aortic beat could be felt by palpation, the retractors were adjusted so that the aperture would admit the fingers of the operator. An incision was made in the aortic sheath, and the aorta exposed, care being taken to avoid unnecessary injury to the aortic plexus, then a blunt probe was cautiously introduced around the aorta at a level between the adrenolumbar and iliolumbar arteries. The aorta was thus isolated from the inferior vena cava and the adjoining nerves. The probe was followed by an aneurysm needle threaded with no. 30 black cotton thread, a double ligature was drawn under the aorta, and both threads tied twice.

This was found to be the critical point in the operation for two reasons: first, because of the danger of including some nerve or blood vessel in the ligation, and secondly, because of sudden strain upon the heart, especially if some undiagnosed cardiac or vascular disturbance was present. Death may result from this cause. In one case, the operation progressed normally until the aorta was tied. At this point the heart stopped beating and respiration ceased. All attempts to resuscitate the animal failed. On autopsy the pericardium was found to contain about a thimbleful of straw-colored serous fluid, indicative of pericarditis.

In closing the wound the layers of muscle were sutured separately with no. 00 catgut. The subcutaneous fat and fascia were also sutured as a separate layer. The skin wound was closed with a modified mattress suture, employing a single thread. This type of suture was used because it is easy to remove if necessary.

The wound was painted with tincture of iodine and dusted with a bismuth-formic-iodide drying powder (Mulford's B-F-I powder). No bandages were applied, as the cats would not tolerate them and in struggling to free themselves they often succeeded in opening and infecting the wound. Immediately after the operation, the femoral pulse was tested by palpation in both hind limbs, its absence being indicative of a successful ligation.

3. *Postoperative testing methods.* In order to ascertain the degree of recuperation following the ligation, the cats were tested in the following manner: Two hours after the operation and at twenty-four-hour intervals, the animals were allowed to walk a distance of 25 feet. When this distance was successfully covered without hesitation, the cat was considered to have established sufficient collateral circulation for the proper functioning of the hind limbs.

4. *Killing, color-injecting, and preservation of anatomical materials.* All the cats in this investigation were killed with chloroform, embalmed with a solution of alcohol, formalin, phenol, and glycerin, and injected with a color mass consisting of black insulating varnish in alcohol. All the specimens were injected at a uniform air pressure of 15 pounds.

DATA

A. Experimental data

1. *Immediate results of ligation.* The general results of ligating the abdominal aorta are: a) cessation of the femoral pulse; b) lowering of the temperature of the affected parts; c) asphyxiation of the affected nervous elements.

a. Cessation of the femoral pulse. Immediately after successful ligation of the abdominal aorta, the femoral pulse

disappears. This condition continues until the diameter of the collateral vessels has increased sufficiently to bring about the gradual reestablishment of the pulse. However, the reappearance of a perceptible femoral pulse is always considerably antedated by the return of functions of the hind limbs. This indicates that the absence of the femoral pulse is not an accurate index of inadequate blood supply.

b. Lowering of the temperature in the affected parts. Exact temperature changes were not recorded in this experiment. However, it was noticed that the temperature of the affected part closely approached the temperature of the room, as determined by feeling the hind limbs at short intervals. It was noted, also, that the distal portion of the hind limbs was the first to show a drop in temperature. There was a gradation in warmth at the different levels of the limb, the warmest portion being the closest to the body.

c. Asphyxiation of the affected nervous elements. In the cat the loss of sensation due to asphyxiation of the nervous elements in the posterior limbs precedes paralysis. The affected limbs were tested for sensation by pinching the toes. Sensation was always present in cats able to walk successfully. It was absent in the paralyzed cats.

2. *Results of ligation in the male.* The typical male response to ligation of the abdominal aorta was not a complete loss of the use of the posterior limbs, but rather a retardation in the normal walking reaction. At the first test, two hours after the operation, the cat invariably refused to walk more than 3 feet without stopping to rest. While walking, the forward movement of each hind limb was retarded. This caused the posterior portion of the body to lean in the direction of the slow limb. Occasionally male cats would have this 'stagger' sufficiently pronounced to cause them to fall upon their haunches. The placing of the feet upon the floor at this time was very inaccurate, resulting in short steps and side-stepping. This may have been due to the partial loss of tactile sensibility as well as to the probable loss of proprioceptive sense.

Table 1 shows that generally the recovery time for the male group was twenty-four hours and over seventy-two hours for the female group. These figures are based upon the twenty-four-hour observation periods.

TABLE 1
The time of recovery for male and female cats after ligation of the abdominal aorta

MALE		FEMALE	
Cat no.	Recovery time, hours	Cat no.	Recovery time, hours
8	24	1	96
11	48	2	72
9	72	3	96
B4	36	10	72
T1	24	12	72
T2	24	14	120
3	24	29-2	132
R1	24	B3	96

A typical example of the reactions of a male cat to ligation of the abdominal aorta is given in the following protocol:

May 9, 1930. Operation begun at 11 A.M. and lasted for thirty-three minutes. Considerable bleeding at the severed cutaneous vessels immediately following ligation of the aorta. When tested two hours later, cat showed a pronounced 'stagger.' No femoral pulse palpable, and hind limbs cold to the touch.

May 10, 1930. When tested again at 9 A.M., the walking reactions were considered normal. No femoral pulse palpable, though hind limbs were warm. Animal killed, embalmed, and color injected.

3. *Results of ligation in the female.* The response on the part of the female was quite different (table 1). As in the male, the femoral pulse ceased upon ligating the aorta and the hind limbs subsequently became cold to the touch. Paralysis usually followed immediately upon ligation and subsequent to recovery from ether and continued for a varying number of hours, in most cases not more than twenty. The recovery from the paralysis was gradual and progressed from the proximal to the distal levels of the hind limbs. Observations and tests were made two hours after the operation and at twenty-four-hour intervals, as in the male.

A typical example of the reactions of a female cat to ligation of the abdominal aorta is given in the following protocol:

June 12, 1928. Operation performed in the morning and lasted thirty minutes. Uneventful. No femoral pulse, and hind limbs cold at end of operation. After recovery from anesthesia, walking reactions were quite sluggish, almost paralyzed. Cat would walk only about a foot.

June 14, 1928. Wound painful to the touch. Walking sluggish and slow. No femoral pulse palpable.

June 15, 1928. The cat could walk quite normally. No femoral pulse.

June 16, 1928. No femoral pulse yet palpable, though the cat walked quite easily. Killed, embalmed, and color injected.

In general, the females presented a more pronounced response than the males. Paralysis of the hind limbs caused the dragging of these members unless the body was the animal was supported by the attendant. As a rule, the inhibition of nervous control was greater in the female, giving a complete loss of sensation when the hind limbs were pinched. If walking movements could be made under these circumstances, they were very inaccurate and of short duration. Subsequent to the onset of return to normal, the female exhibited the same reactions as described for the male.

Working upon the hypothesis that the presence of the mammae and the ovaries in the respective collateral channels was responsible for the difference in response time between the male and female cats, the following operations were performed:

The superficial branches of both superior epigastric arteries as well as the spermatic were ligated in two male cats. The superficial superior epigastrics, which supply the mammae in the female, were tied as they branched from the superior epigastrics in the muscles just posterior to the last costal cartilage. The spermatic arteries, which correspond to the ovarians in the female, were ligated through a ventral abdominal incision. The aorta was tied in the usual manner, though the operation in these two cases was intraperitoneal.

The results of such an operation indicate that the male cats thus operated upon show typical female response, as shown in the following protocols:

Cat no. R2, male, May 28, 1930. Operation begun at 10.50 A.M. and continued for forty minutes. Both superficial branches of the superior epigastric, spermatics, and the aorta ligated. At 4 P.M. there were typical female reactions to aortic ligation. There was partial paralysis and staggering when attempting to walk. No femoral pulse palpable and the hind limbs were cold.

May 29, 1930, 9 A.M. No tendency to walk whatever. When forced to walk, cat is very deliberate in the placing of its feet. A slight tendency toward 'knuckle walking' is noticed. No femoral pulse perceptible. At 1 P.M. the animal walked with less coordination than before. There is now practically complete paralysis. The animal is unwilling to move.

May 30, 1930, 1.30 P.M. The cat walks better, but still staggers. It arches its back with difficulty. There is no femoral pulse as yet, but sensation is present when toes are pinched.

May 31, 1930, 3 P.M. The animal walks quite unsteadily, making each step with a deliberate placement of the foot. There is very slight side-sway. No femoral pulse palpable.

June 2, 1930, 9 A.M. The animal walks with only a slight stagger. It will not walk far, however, without resting. No femoral pulse yet.

June 3, 1930. The animal walked the entire 25 feet several times in a normal manner. Killed, embalmed, and color injected.

Cat no. T5, male, June 8, 1930. Operation begun at 9.30 A.M. and lasted forty-three minutes. The operation was essentially the same as for cat no. R2. No femoral pulse palpable after the ligation, and complete paralysis soon set in. 7.30 P.M. The animal cannot walk, but can stand up. Hind limbs cold from the knee down. No femoral pulse palpable.

June 9, 1930, 9 A.M. The animal can walk with extreme difficulty. The feet are cold and there is no femoral pulse palpable.

June 10, 1930, 9 A.M. The animal walks slowly and with great difficulty. No better than on the 9th. No femoral pulse palpable.

June 11, 1930, morning. The animal walks slower and with uncertainty. No femoral pulse perceptible.

June 12, 1930, evening. The animal walks very well. There is no femoral pulse palpable. Killed, embalmed, and color injected.

4. *Unusual results.* Three cats, not included in table 1, presented unusual prolongation of paralysis: One female failed to walk normally for thirty-two days; another female

for twenty-one days; and a male for forty-nine days. Two possible causes of these unusual results are the disturbance of the aortic plexus and interference with the circulation to the lumbar spinal cord through the lumbar arteries.

In order to test the effect of injury to the aortic plexus attending ligation of the aorta, the following operation was performed upon a male and a female cat: The usual operative approach was made as for the routine ligation, but care was taken to disrupt the aortic plexus as much as possible. The male recovered in three days, and the female in six days. There is, therefore, but slight departure from the usual reactions to aortic ligation in these two cats.

The effect of partially disturbing the blood flow to the lumbar spinal cord was tested in the following manner: A male cat was placed dorsocumbent on the operating table and a median ventral incision was made through the abdominal wall in the linea alba. The incision was carried through the peritoneum. The viscera were retracted so that the aorta was visible from the adrenolumbar arteries to its bifurcation. The aortic sheath was carefully opened, and the sixth and seventh pairs of lumbar arteries were ligated as well as the left fifth. As the animal at this point showed signs of surgical shock, the other fifth lumbar was not tied, but the aorta was ligated in the usual manner. Paralysis of the hind legs set in one-half hour after ligation and the cat died one and one-half days after the operation. On autopsy the limbs showed signs of decomposition.

5. *Additional observations.* In all the cats which were subjected to aortic ligation none developed gangrene. There were, however, two cases of infection in the hind limbs following the operation. In one cat, an infection of the synovial sac of the right ankle developed four days after the operation. Its cause could not be determined with certainty, but it may have been due to tight bands that held the cat on the operating table, or to rat bites. The sac was drained repeatedly and the cat eventually recovered. Another cat became infected on the dorsal surface of her toes because of her prolonged use

of 'knuckle walking,' in which the cat does not extend the digits and assume true digitigrade walking, but keeps the toes flexed—walking on the knuckles. These are the only instances of infection of the posterior extremities and were not directly due to the operation or to the ligation.

Though the results of ligating the aorta in the cat are followed by conditions which demonstrate the ability of the male to establish an adequate collateral circulation sooner than the female, a general application of this principle would require further experimental proof from other animals. Therefore, ten albino rats were subjected to an operation similar to that on the cat and tested for their recovery time. The result of this experiment shows that female albino rats recover from aortic ligation in about one and one-half days, while the males recover in about one-tenth of a day. The albino rat, therefore, exhibits a reaction to aortic ligation similar to that of the cat.

B. Anatomical data

Examination of prepared specimens of the operated cats reveals the following arterial anastomosis:

1. *Normal arterial anastomosis.* Of the arterial anastomosis capable of establishing collateral response all the cats uniformly exhibited the following:

a. *Adreno-iliolumbar anastomosis.* The adrenolumbar and iliolumbar arteries unite by caudal and cranial branches, respectively. This provides the best collateral channel in the region of the aorta.

b. *The last lumbar with the inferior epigastric.* The last pair of lumbar arteries arise from the aorta at the level of the origin of the external iliac. They divide into two branches, a dorsal and a lateral, the latter giving off a branch which anastomoses with the inferior epigastric, a branch of the profunda femoris.

c. *The sixth lumbar with the last (seventh) lumbar.* The anastomosis of the respective lumbar arteries in the cat permits ligation at almost any level between the adrenolumbar and iliolumbar arteries.

d. The fifth lumbar with the circumflex femoris. The anastomotic branch of the fifth lumbar connects with the circumflex femoralis lateralis after passing through the canal of Hunter along with the femoral.

e. The spermatic with the hypogastric. In the male the cremasteric branch of the spermatic anastomoses with the corresponding branch of the deep epigastric. In the female, however, the ovarian passes through the ovary to anastomose with the uterine.

f. The internal mammary with the inferior epigastric. This channel, in the male, provides a considerable collateral blood flow. In the female, the vessels break up and ramify through the mammae.

g. The superior hemorrhoidal with the middle hemorrhoidal. The superior hemorrhoidal is a single artery which arises from the inferior mesenteric, passes caudad along the descending colon and rectum, and anastomoses with the middle hemorrhoidal, a branch of the hypogastric.

3. *Anomalous arteries.* An anomalous artery has been found twice in the cats used in this investigation. A single artery arose just cephalad of the inferior mesenteric and accompanied the aorta to anastomose with the vesicular branch of the anterior division of the internal iliac.

4. *New arterial outgrowths.* In cats which, because of the slow return to normal, were permitted to live almost a year in the laboratory new arteries were found that grew out from the aortic wall to anastomose around the site of ligation.

DISCUSSION

One of the results of totally ligating the main artery to a certain portion of the body is the immediate reduction in the flow of blood to that part. This causes an exhaustion of the oxygen content of the blood in the affected part, and, in turn, produces local asphyxiation of the tissues. This phenomenon is more noticeable in the nervous and muscular elements, and if it is not relieved almost immediately by the collateral response, it will lead to a state of paralysis.

In the average cat the establishment of an adequate collateral circulation, after ligation of the aorta, is rapid; and the results attending the ligation seldom last long enough to produce temporary paralysis of the hind limbs. In the female the collateral cross-section is not always adequate, and temporary paralysis sometimes results, but it is not sufficiently prolonged to cause gangrene of the hind limbs.

Nevertheless, three cats of this series have exhibited unusually long periods of paralysis with subsequent slow return to normal. This departure from the usual rate of recovery was thought to be due either to inflammation, inclusion of nervous tissues within the ligation, or to injury to some of the lumbar arteries, more particularly the fifth, sixth, and seventh pairs.

It was found that there was no evidence of infection at the site of ligation in any of these three cats. This shows that inflammation can be eliminated as a possible cause for this retardation.

The result of experimentally testing the importance of maintaining the continuity of the aortic plexus shows that the cats, male and female, subjected to this test recovered—the male in three days and the female in six days. This lapse of time for the respective sexes shows but a slight departure from the usual results of aortic ligation. We may conclude that injury to the aortic plexus at this point is not of great importance in the return of the normal walking reaction.

The result of experimentally injuring the lumbar arteries demonstrates that complete paralysis of the posterior limbs, under this condition, takes place within one-half an hour after the operation, and continues in an aggravated form far beyond the usual time of recovery. Therefore, it seems that, in the cases cited above, prolonged recovery time was due in part to disruption of the blood supply from the lumbar arteries to the spinal cord.

The return of normal walking of the hind limbs, following ligation, is directly dependent upon the collateral channels. It has long been recognized that there are certain by-paths for the blood if particular arteries are occluded. Such

bypaths have been called 'collaterals,' and exist largely in a potential state until the main channel is obstructed.

Guthrie ('12) describes at length the physical factors involved in the establishment of a collateral circulation. According to him, immediately after ligation of any main artery the capillary pressure falls, bringing about an increase in the velocity of the blood in the accompanying arteries, which will rapidly return the capillary pressure to normal, if the cross-section is sufficiently large. If inadequate, there results a paralysis of the capillaries. This offers a resistance to the blood passage through the tissues. He also believes that there is a possible vasomotor reflex to increase the diameter of the vessels.

In considering the collateral arterial response, the importance of the venous return should not be overlooked. Guthrie ('12) calls attention to the fact that if the main artery and main vein to a limb be severed or included in a ligature, gangrene is the inevitable result. It is essential, therefore, that venous drainage be adequate when the main artery to a part has been ligated. The venous bed is usually capable of this, due to the profuse collateral channels that it possesses.

It has been mentioned earlier in this paper that if a cat with ligated aorta be allowed to continue life for a year or more, new vessels grow out around the site of ligation to form new collateral channels. The formation of new collateral outgrowths is of little importance to the question at hand because of the time involved in their formation. It is possible, however, that in prolonged collateral response the formation of new vascular channels would facilitate the return of normal use to the affected limbs.

The obvious difference in the anatomical arrangement of the collaterals in the two sexes was the presence of capillary beds in the mammae and the ovaries in the female as compared with the absence of such beds in the male. It was noted that the superficial branches of the superior epigastric in the male were continuous with the superficial inferior epi-

gastric. A similar condition between the spermatic and cremasteric arteries also exists. In the female, however, the superficial superior epigastric breaks up in the mammae, so that only smaller arterioles are continuous with the branches of the superficial inferior epigastric. The ovaries likewise present similar connections between the ovarian and the uterine arteries. Here, then, is definite evidence of a less direct means of anastomosis in the female. To reproduce an analogous condition in the male, the superficial branch of both superior epigastrics, the spermatics, and the aorta were ligated in two males. Both males thus operated upon gave typical female response. These results permit a general statement to the effect that the difference between the male and female collateral response, favoring the male, is due to the mechanical effect of the reduction of the collateral channels to small vessels as they pass through the mammae and ovaries, thus retarding the flow of blood.

CONCLUSIONS

1. Permanent ligation of the abdominal aorta in the cat, between the origin of the adrenolumbar and the iliolumbar arteries, is attended by only temporary incapacity of the hind limbs.
2. The cats thus operated upon are able to walk before the return of a palpable femoral pulse.
3. Prolonged and complicated incapacity of the hind limbs may be caused by partial interference with the blood supply to the lumbar portion of the spinal cord through injury to the lumbar arteries.
4. Injury to the aortic plexus has no perceptible influence on the results of aortic ligation.
5. Males, on the average, recuperate more rapidly than females after ligation of the abdominal aorta between the adrenolumbar and iliolumbar arteries.
6. This difference is due to a purely mechanical condition. In the female the mammae and ovaries interpose a vascular bed which interferes with the rapid establishment of an adequate collateral response.

7. Recovery, in both sexes, is quick enough and collateral channels abundant enough to offset any tendency toward gangrene in the affected parts.

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PROCEEDINGS OF THE AMERICAN ASSOCIATION
OF ANATOMISTS

FORTY-SEVENTH SESSION

*Northwestern University Medical School, Chicago, Illinois,
April 2, 3, and 4, 1931*

THURSDAY, APRIL 2, 1931

10.00 a.m. The Forty-seventh Session of the American Association of Anatomists was called to order by Vice-President Edgar Allen, who announced the appointment of the following committees:

Auditing Committee: Professor Wm. F. Windle, Chairman; Professor O. F. Kampmeier.

Nominating Committee: Professor C. M. Jackson, Chairman; Professor Charles R. Stockard, Professor P. E. Smith.

The remainder of the session was devoted to the presentation of scientific papers.

FRIDAY, APRIL 3, 1931

12 noon. Association Business Meeting, with Vice-President Edgar Allen presiding. The Secretary reported that the minutes for the forty-sixth session were printed in full in *The Anatomical Record*, volume 45, no. 4, pp. 293-298, inclusive. On motion, seconded and carried, the minutes of the forty-sixth session were approved by the Association as printed in *The Anatomical Record*.

Report of the Treasurer: The Treasurer made the following report for the year 1930:

Balance on hand, December 31, 1929 (last audit of accounts)	\$1,161.09
Receipts from dues and abstracts, 1930	549.92
Interest on deposits:	
4/24/30	\$ 8.67
5/29/30	2.17
11/28/30	19.23
	30.07
Total credits	\$1,741.08
Expenditures for 1930:	
Postage	\$ 40.00
Wistar Institute (abstracts, Anat. Rec.)	81.00
Printing and stationery	41.34
Secretarial services	50.00
Local committee on arrangements (Charlottesville)	150.00
Traveling expenses—Secretary (Charlottesville)	25.96
Programs—International Anatomical Congress	18.50
Programs—46th Session	75.00
	481.80
Total expenditures	481.80
Balance on hand, December 31, 1930	\$1,259.28
Deposited in the name of the American Association of Anatomists, in the Lincoln-Alliance Bank & Trust Co., Rochester, New York.	

Report of the Auditing Committee: Professor Wm. F. Windle reported for the Auditing Committee as follows: "The undersigned have examined the books of the Secretary-Treasurer for the year 1930, and have found his statement to be correct."

[Signed] WM. F. WINDLE,
OTTO F. KAMPMEIER.

On motion, duly seconded, the reports of the Treasurer and of the Auditing Committee were accepted and adopted.

Election of officers: Professor Harvey O. Jordan, Chairman of the Committee on Nominations for 1931, placed before the Association the following nominations for office:

For Members of the Executive Committee: Professor Paul S. McKibben, of the University of Southern California, and Professor Edward F. Malone, of the University of Cincinnati.

On motion, seconded and carried, the Secretary was instructed to cast a ballot for the election of the above named, with terms of office as specified in the Constitution of the Association.

New Members. The Secretary presented the following names recommended by the Executive Committee for election to membership in the Association:

- ASDELL, SYDNEY ARTHUR, Ph.D., Assistant Professor in Charge of Physiology of Reproduction, State College of Agriculture, *Cornell University, Ithaca, N. Y.*
- VON BONIN, GERHARDT, M.D., Associate in Anatomy, University of Illinois, College of Medicine, *1817 West Polk St., Chicago, Ill.*
- CHARLES, CECIL M., A.B., M.A., Ph.D., Assistant, Department of Anatomy, *Washington University School of Medicine, St. Louis, Mo.*
- COLE, ARCH EVAN, A.B., Ph.D., Instructor, Anatomy Department, *University of Louisville, Louisville, Kentucky.*
- COLE, HAROLD H., Ph.D., Assistant Professor of Animal Husbandry, University of California, and Assistant Animal Husbandman, *Experiment Station of the College of Agriculture, Davis, Calif.*
- EDWARDS, LINDEN F., Assistant Professor of Anatomy, Ohio State University College of Medicine, *Hamilton Hall, Columbus, Ohio.*
- FLEXNER, LOUIS BARKHOUSE, S.B., M.D., Instructor in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- FRASER, DORIS A., Ph.D., Assistant in Anatomy, *University of Pennsylvania School of Medicine, Philadelphia, Pa.*
- GEORGE, RUGGLES KERR, B.A., M.B., D.P.H., Assistant in Department of Anatomy, *University of Toronto, Toronto 5, Canada.*
- GRODINSKY, MANUEL, B.Sc., M.D., Assistant Professor of Anatomy, University of Nebraska, College of Medicine, *42nd and Dewey Ave., Omaha, Neb.*
- HALL, BYRON E., Teaching Fellow in Anatomy, *University of Minnesota Medical School, Minneapolis, Minn.*
- INGRAM, W. R., Ph.D., Instructor in Neurology, Northwestern University Medical School, Institute of Neurology, *303 E. Chicago Ave., Chicago, Ill.*
- KRAFKA, JOSEPH, JR., Ph.D., Associate Professor of Anatomy, Medical Department, *University of Georgia, Augusta, Ga.*
- LUCAS, MIRIAM SCOTT, Ph.D., Instructor in Cytology, *Washington University School of Medicine, St. Louis, Mo.*
- McFALL, CLAUDE M., Ph.D., Assistant Professor of Biology, School of Histology and Embryology, *University of Virginia, Charlottesville, Va.*
- McKINNEY, ROSCOE L., Ph.D., Professor of Anatomy, *Howard University, Washington, D. C.*

- PRATT, JEAN PAUL, M.D., Surgeon in Charge of Division of Gynecology and Obstetrics, *Henry Ford Hospital, Detroit, Mich.*
- ROGERS, JAMES B., A.M., M.D., Assistant Professor of Anatomy, *University of Louisville, School of Medicine, Louisville, Kentucky.*
- SHEARER, EDWIN M., Ph.D., Instructor in Anatomy, *New York University and Bellevue Hospital Medical College, Twenty-sixth St. and First Ave., New York City.*
- WILLIAMS, GEORGE D., A.B., A.M., M.D., Ph.D., Assistant Professor of Anatomy, *Washington University School of Medicine, St. Louis, Mo.*
- WILLIAMS, ROY GRIFFIN, A.B., A.M., M.D., Department of Anatomy, *University of Pennsylvania School of Medicine, Philadelphia, Pa.*
- WILLIAMS, STEPHEN CULVER, Ph.D., Instructor in Anatomy, *Medical Department, University of Pennsylvania, Philadelphia, Pa.*
- ZECHEL, GUSTAV, M.D., Associate in Anatomy and Surgery, *University of Illinois College of Medicine, 1817 West Polk St., Chicago, Ill.*

On motion, the Secretary was instructed to cast a ballot for all candidates proposed by the Executive Committee.

Members Resigned: The Secretary announced the following names of members who have resigned since the last meeting:

JOHN CAMERON.
W. F. HEMLER.

F. W. STEWART.
HENRY BAYON.

Place of Next Meeting. It was announced that the Executive Committee had decided to hold the forty-eighth annual session of the Association at the College of Physicians and Surgeons, Columbia University, New York City, on the three days immediately preceding the next Easter (March 24, 25, and 26, 1932).

Dues for the Year 1931. The Executive Committee recommended that the dues for the year 1931 be set at \$1.00 per member. This recommendation of the Executive Committee was adopted by the Association.

Payment to Local Committee. In accordance with the policy established by the Association at the forty-third session, it was moved and seconded that the Treasurer be authorized to pay the sum of \$150.00 from the general funds of the Association to the Local Committee on Arrangements at the Northwestern University Medical School for the purpose of defraying the cost of entertainment of the Association during the present forty-seventh session.

Representatives of Association. On recommendation of the Executive Committee, the Association voted to continue the following appointments of representatives: Delegates to the Council of the American Association for the Advancement of Science, Professor J. Playfair McMurrich, Professor Harold D. Senior; representative on the Commission on Standardization of Biological Stains, Professor Harvey E. Jordan; representative to the Division of Medical Sciences of the National Research Council, Professor J. Parsons Schaeffer; delegate to the Union of Biological Societies, Dr. Henry H. Donaldson.

Committee on International Anatomical Congress. It was stated on behalf of the Committee that its final report was published in *The Anatomical Record*, volume 45, supplement of February 25, 1931. On motion by Professor J. L. Bremer, of the Committee, duly seconded and carried, the Treasurer was directed to transmit to the chairman of the local committee of the International Congress at Amsterdam the sum of \$100 as the contribution of the American Association of Anatomists toward the expenses of the Congress.

New Business. Dr. H. H. Donaldson reported to the Association regarding the present need of greater support, by the Association, of the publication *Biological Abstracts*. On motion by Dr. G. L. Streeter, duly seconded and carried, the Treasurer was directed to transmit to the Union of Biological Societies the sum of \$100 as evidence of the interest of the Association in the *Biological Abstracts*.

The Secretary reported the submission, for action at the forty-eighth session, of two amendments to the Constitution providing for the addition of a Second Vice-President to the present officers of the Association.

After a brief discussion regarding the advisability of instituting a form of associate membership in the Association, Professor L. B. Arey and Professor B. H. Willier were appointed by the Vice-President as a committee to consider the matter and to report upon it to the Association.

On motion, the business session then adjourned.

SATURDAY, APRIL 4, 1931

12 noon. Concluding business session. A short business meeting followed the morning scientific sessions. The Secretary reported that there was no unfinished business.

Resolution of Appreciation. Professor F. L. Landacre presented the following resolution:

Resolved: That the members of the American Association of Anatomists desire to express to the authorities of Northwestern University Medical School and to our colleagues of the Departments of Anatomy and Neurology, our warm appreciation of the cordial hospitality and effectiveness with which the forty-seventh session has been welcomed. It has been a real pleasure to inspect the unique plan of the Medical School in operation and to observe the facilities for research in anatomy. We are under deep obligation to the Local Committee for their considerateness and hospitality.

Moved, seconded, and unanimously voted.

On motion, the session adjourned.

GEORGE W. CORNER,

Secretary of the Forty-seventh Session of
the American Association of Anatomists.

AMERICAN ASSOCIATION OF ANATOMISTS

OFFICERS AND LIST OF MEMBERS

Officers

<i>President</i>	HERBERT M. EVANS
<i>Vice-President</i>	EDGAR ALLEN
<i>Secretary-Treasurer</i>	GEORGE W. CORNER

Executive Committee

For term expiring 1932.....	EDWARD A. BOYDEN, CHARLES C. MACKLIN
For term expiring 1933.....	HAROLD S. BURR, CARL G. HARTMAN
For term expiring 1934.....	LESLIE B. AREY, SAMUEL R. DETWILER
For term expiring 1935.....	PAUL I. MCKIBBEN, EDWARD F. MALONE

Delegates to the Council of A. A. A. S.

J. PLAYFAIR McMURRICH
HAROLD D. SENIOR

Representative to the National Research Council

J. PARSONS SCHAEFFER

Commission on Standardization of Biological Stains

HARVEY E. JORDAN

Delegate to Union of Biological Societies

HENRY H. DONALDSON

Committee on Nominations for 1932

C. M. JACKSON, *Chairman*
CHARLES R. STOCKARD P. E. SMITH

HONORARY MEMBERS

S. RAMÓN Y CAJAL.....	Madrid, Spain
A. NICHOLAS.....	Paris, France

MEMBERS

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- DE GARIS, CHARLES F., A.B., M.D., Ph.D., Associate in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- DETWILER, SAMUEL RANDALL, Ph.B., A.M., Ph.D. (Ex. Com. '30-), Professor of Anatomy, Department of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*
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THE INDUCTION OF PSEUDOPREGNANCY IN THE RAT BY MEANS OF ELECTRICAL STIMULATION

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ONE FIGURE

The condition of pseudopregnancy was first induced artificially, in the rabbit, by Ancel and Bouin ('09), by mating a vasectomized buck with a normal female. Long and Evans ('22) and Slonaker ('29) mated normal female rats with vasectomized male rats and provoked pseudopregnancy. This condition is manifested, in the rat, by the typical phenomena of early pregnancy, namely, a prolonged leucocyte vaginal smear, reduced spontaneous activity, increased body weight, increased food intake, and slight mammary-gland development (Slonaker, '29).

Long and Evans ('22) produced the condition of pseudopregnancy in the rat by mechanical stimulation of the cervical canals of the uterus with a glass rod when the animal was in stage 1, 2, or 3 of the oestrous cycle as designated by them. Their work has been corroborated by Wang ('23), Slonaker ('29), Meyer, Leonard, and Hisaw ('29), and others. Slonaker found, that although he used a wide range of types and sizes of glass rods, which he manipulated with great care lest he injure the vaginal mucosa, the percentage of cases of pseudopregnancies was very much lower than that recorded by Long and Evans. Sterile copulation yielded 100 per cent results according to Slonaker's reports. He suggests the secretion of a hormone by the vaginal mucosa or by the endometrium as a possible stimulus to the condition. Meyer,

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Leonard, and Hisaw found that cervical stimulation with a glass rod in the normal unanaesthetized rat provoked a percentage of cases of pseudopregnancy equal to that reported by Long and Evans—about 70 per cent. In anaesthetized animals, however, the cases were 35 to 45 per cent fewer, varying with the type of anaesthesia used. Hisaw and his coworkers are inclined to believe that the initial stimulus in inducing pseudopregnancy is a nervous rather than humoral one. Fee and Parkes ('30) reported that the administration of local anaesthesia to the vagina of rabbits was ineffective in decreasing the libido, or in altering the number of cases of pseudopregnancy.

In an approach to the study of the mechanism of pseudopregnancy, a method has been devised which induces that condition in the rat, so that the exact time of induction can be recorded, and yet provokes a higher percentage of cases than the glass-rod procedure. The method consists of electrical stimulation of the cervix of an animal when the vaginal smear is in the nucleated cell stage, the mixed nucleated and cornified cell stage, or in the dry cornified cell stage. A small copper electrode which is connected to an induction coil, operated by two dry-cell batteries, is inserted in the vagina, and with the aid of a speculum and speculum mirror, the tips of the wires are placed at the opening of the cervix. The circuit is then closed, and a light electrical shock is administered; the key is quickly released and the electrode removed. The current was never great enough to cause any injury as burning or bleeding. Daily vaginal-smear records of all the animals were kept.

In an original trial run on five albino rats (series A), which were five months old and ranged in weight from 196 to 203 grams, three became pseudopregnant. A second series consisted of forty-two normal, nulliparous females ranging from five to thirteen months of age and from 150 to 252 grams in weight. The average age was 7.7 months, and the average weight was 186.0 grams. Twenty-eight animals were used experimentally and the remaining fourteen were used as con-

trol. Table 1 shows the general number of animals and percentages of pseudopregnancy of the series of experimental animals and controls. Series A was the original trial run before the technic of stimulation was finally developed. The second series, B, resulted in 82 per cent pseudopregnancies. This is higher than previous records of percentage of cases arising from mechanical stimulation. The percentage calculated from data presented by Long and Evans (p. 80, '22), was about 68 per cent; Leonard, Meyer, and Hisaw record a maximum of 72 per cent; Slonaker reports that his results were very much lower than the Long and Evans data showed. The results of the control animals which received the same treatment as the experimental animals except that no electrical current was administered were somewhat surprising, in

TABLE 1
Showing number of animals and percentages of pseudopregnancy

SERIES	NUMBER OF ANIMALS	PSEUDO-PREGNANCIES	FAILURES	PERCENTAGE PSEUDO-PREGNANCY
A (trial run)	5	3	2	60.0
B	28	23	5	82.1
C (controls)	14	0	14	0.0

that they were all negative. It was originally supposed that the bare electrode might function somewhat similarly to the glass rod, but since no cases of pseudopregnancy were induced, the difference in shape, the lack of movement of the electrode, or in the lapse of time that the electrode remained at the cervix demonstrated the inadequacy of the stimulus.

Table 2 presents data on the relationship of frequency of pseudopregnancy to phase of oestrous cycle at the time of stimulation. It is especially noteworthy that the greatest frequency of pseudopregnancy occurred in animals which were stimulated electrically at that phase of their oestrous cycles when the vaginal smear consisted of epithelial and cornified cells.

The average length of pseudopregnancy was 14.03 days. The minimum was nine days, and the maximum was twenty-

one (fig. 1). These results check with those reported by Slonaker (seven to nineteen, 14.53 average) and Long and Evans (eight to twenty-three, 14.38 average).

While the above method adds little more than another means of attack to the problem, nevertheless the fact cannot be disregarded that the mechanism is not similar in its action to that of the glass rod as suggested by Long and Evans, namely, as analogous to the copulation plug of the male.

TABLE 2

Showing relationship of frequency of pseudopregnancy to phase of oestrous cycle at time of stimulation

CYCLE PHASE	TOTAL NUMBER OF ANIMALS	PSEUDO-PREGNANCIES	FAILURES	PERCENTAGE PSEUDO-PREGNANCY
0	14	10	4	71.5
0 ₁	12	11	1	91.8
Cornified	7	5	2	71.5

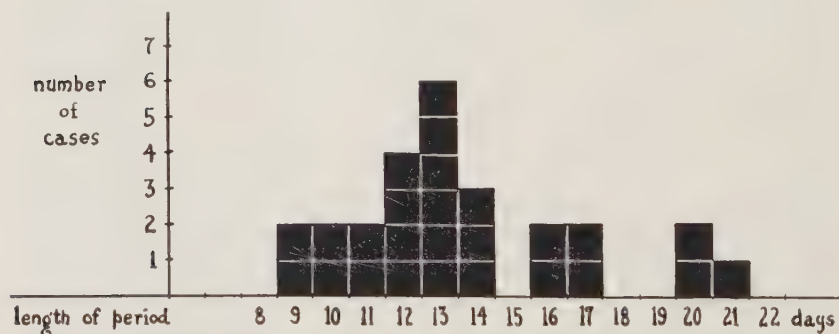


Fig. 1 Graph showing relation of length of pseudopregnancy and number of cases.

SUMMARY

1. A method of electrical stimulation of the cervix is described which induces a greater percentage of cases of pseudopregnancy in rats than other mechanical means.

2. The greatest frequency of pseudopregnancy occurs in animals which are stimulated when they are in 0₁ stage of the oestrous cycle.

3. The average length of pseudopregnancy was 14.03 days.

The author gratefully acknowledges his indebtedness to Dr. Earl T. Engle for the suggestion of the problem and for his invaluable criticism.

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FAILURE TO INDUCE OVULATION IN THE GUINEA- PIG BY INTRAVENOUS INJECTION OF THE URINE OF PREGNANCY

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It was discovered in 1927 by Zondek and Aschheim that the urine of pregnant women contains a substance which when injected into animals produces certain effects similar to those produced by the gonad-stimulating hormone of the anterior lobe of the hypophysis. Among these effects was the production of ovulation in immature rats and mice by repeated injections of the urine from pregnant women. In 1929, Friedman made the discovery that in rabbits a single intravenous injection of the urine of pregnancy is sufficient to induce ovulation. This finding was confirmed and further investigated by the present writer (Jares, '30), and employed as a means of diagnosing pregnancy, according to the suggestion of Friedman.

It should be recalled that rabbits do not ovulate spontaneously (except, possibly, as a rare exception to the rule). If kept in isolation, ovulation occurs normally only when induced by mating. In this respect the rabbit is notably different from most other mammals so far investigated, including the other small rodents commonly used in the laboratory, all of which undergo regular oestrous cycles with spontaneous ovulation.

The ability to induce ovulation without copulation and therefore without the complications of pregnancy, and to repeat at will the induction of ovulation with the resulting corpora lutea in the same animal, has already proved to

be of great service in experimental work, as is evidenced by Corner's ('30) application of this discovery as a means of disproving that mammary-gland proliferation and secretion are due to the direct influence of corpora lutea in the rabbit. It therefore becomes most important to know whether the striking effect thus produced in the rabbit can be obtained also in those animals which ovulate spontaneously at periodic intervals.

In order to answer this question, experiments were undertaken upon guinea-pigs. In this species oestrus, accompanied by ovulation, recurs at intervals of about fifteen to sixteen days.

Adult, virgin guinea-pigs were observed as to the normalcy and regularity of their oestrous cycles by noting the swelling of the vulva and the rupture of the vaginal closure membrane at the time of oestrus. The vaginal wash technique was employed during the oestrous period to define quite exactly the time of ovulation: the appearance of large numbers of nucleated epithelial cells characterizes this occurrence (Stockard and Papanicolaou, '17).

From three to ten normal oestrous cycles were observed in each animal before injection. In addition, sterile exploration of the animal previous to the administration of urine was performed in five cases. The usual protruding corpora lutea and normal-sized follicles were noted at these control observations of the ovaries.

The experiments were performed as follows: The animal was placed under ether anaesthesia; an incision was made in the ventral neck region and the jugular vein exposed. The quantity of urine injected into the vein ranged from 1 to 5 cc. It was found that approximately 5 cc. was the maximal dose that could be given without endangering the life of the animal. The injections were made with freshly voided urine obtained from women at various stages of pregnancy (table 1). The potency of these urine specimens as regards the gonad-stimulating substance was tested by intravenous injection into rabbits, and in each case a positive ovulation test was

observed, even when a very much smaller quantity (for example, 0.05 cc. of the urine from a woman about 2.5 months pregnant) was employed.

At autopsy, varying from one to eight days after injection (except in those cases where the animals were allowed to survive in order to ascertain any possible effect of the urine on the oestrous cycle), the genital organs were examined

TABLE 1

DAYS AFTER OVULATION WHEN INJECTED	DAYS AFTER OVULATION WHEN EXPLORÉD* OR AUTOPSED	GUINEA-PIG NO.	NUMBER OF NORMAL OESTROUS CYCLES OBSERVED BEFORE INJECTION	NUMBER OF NORMAL OESTROUS CYCLES OBSERVED AFTER INJECTION	QUANTITY OF URINE INJECTED (CC.)	DURATION OF PREGNANCY OF DONOR OF URINE (MONTHS, APPROXIMATELY)
1	5	11	5		5	2.5
2	3*, 5	8† ¹	4		4	8
3	5	4†	4		3	4
3		15	3	4	1	6
4	6*	5	3	3	2.5	6
4	5	3†	3		2.5	6
5	6*	4	3	1	2.5	6
5	7*	2	3	3	2.5	6
6	10	6	10		5	2.5
9	10*	2†	4	2	3	4
9	11*, 12	7†	4		3	4
9	13	10	6		5	2.5
9		11	3	2	4	2.5
10	13	13	3		5	8
12	13*, 14	1	3		4	8
12	5	14	7	1	5	2.5
14	3	12	5	1	4	2.5

¹ Exploration previous to injection designated by †.

macroscopically, and the ovaries and a portion of the uterus removed and prepared for histological study.

Ovulation did not result from the injections in any animal. Careful microscopical study of the serially sectioned ovaries and sample sections of the uteri revealed no significant difference from control sections. Thanks to the pioneering work of Stockard and Papanicolaou, the age of the corpora lutea

could be ascertained fairly exactly from the ovaries fixed during the various stages of the oestrous cycle. Thus histological confirmation of the ovulation date as noted from the vaginal wash could be made.

As further evidence that the injection of pregnant urine did not induce ovulation, it should be mentioned that in no case did the ovaries possess when examined after the injection a larger number of corpora lutea than normally found in this species, i.e., three or four in the two ovaries. In other words, all the recent corpora lutea observed in the experimental animals were fully accounted for, by both histological and numerical evidence, by the last regular ovulation previous to the injection.

Moreover, no effect on the regularity, time relationship, or general characteristics of the oestrous cycle was observed in guinea-pigs 2, 4, 5, 11, 12, 14, and 15, which were allowed to live from one to four oestrous cycles subsequent to the one in which the injection was made.

These findings indicate that the ovulatory mechanisms of the guinea-pig are in some way different from those of the rabbit. The guinea-pig is therefore not a suitable test animal for the diagnosis of pregnancy in women when a single intravenous dose of the urine is administered.

CONCLUSIONS

1. A single intravenous dose of the urine of pregnant women administered during various stages of the oestrous cycle failed to induce ovulation in adult, virgin guinea-pigs.
2. The regularity, time relationship, and general characteristics of oestrus in the guinea-pig were unaltered by a single intravenous administration of the urine of pregnancy.
3. These findings indicate a somewhat different mechanism for the release of ovulation in the non-spontaneously ovulating rabbit and in the spontaneously ovulating guinea-pig.

I am indebted to Dr. George W. Corner, professor of anatomy, for his kind cooperation, and to the Obstetrical Clinic of Strong Memorial Hospital of the University of Rochester for supplying the urine specimens used.

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OVULATION IN THE PREGNANT RABBIT¹

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Zondek and Aschheim ('28) have reported that a single implantation of 0.05 to 0.1 gram of fresh anterior-lobe tissue into pregnant mice induced follicular growth and ovulation. When this amount of tissue was implanted, there was no interference with the birth mechanism or damage to the foetuses. In their experiments only three pregnant mice were used. In the first mouse, autopsied in about thirty hours after the implantation, living foetuses and normal placentae were found in the uterus. In two other animals foetuses and attached placentae were found in the uteri at autopsy. In all three instances, serial sections of the ovaries and the fallopian tubes were cut. Fresh corpora lutea were found in the ovaries and ova were found in the tubes. The largest number of ova recorded in any one tube was five.

On the other hand, Engle and Mermod ('28) have carried out a much larger series of experiments, using both pregnant rats and mice. In their experiments they used a number of daily implantations, while Zondek and Aschheim used only one relatively large implant. Engle and Mermod reported that implantation of fresh anterior-lobe substance into pregnant rats or mice in the first third of pregnancy prevented nidation or led to the early resorption of the embryos. Treatment begun in the middle third of pregnancy always resulted in resorption or abortion. The same treatment begun in the final third of pregnancy was much less effective in terminating the pregnancy. Part of the treated animals aborted,

¹This work has been aided by a grant from the National Research Council Committee for Research in Problems of Sex.

while others delivered normal litters. In their experiments ovulation in no instance occurred while there were living foetuses in the uteri. Watt ('31) has recently reported a case of ovulation in a pregnant mouse. Copulation and ovulation occurred a very short time before delivery. The animal was killed while in labor. Two living foetuses were found in the uterus. Young corpora lutea were present in the ovaries and ova were found in the tubes. Many large follicles were found in the ovaries. No histological observations on the vagina were reported.

During work on ovulation in the rabbit being carried out in this laboratory, we have on numerous occasions induced ovulation in pregnant rabbits without any apparent harm to the foetuses. The method used in most cases was as follows. The head of sows and the reproductive organs were brought from the slaughterhouse within a few minutes after the death of the animals. Blocks from the ovary, uterus, and vagina were fixed for histological study. The hypophysis was removed and the anterior lobe dissected out. The anterior lobe was carefully weighed and then ground in physiological saline with sterile sand. The mixture was centrifuged for thirty minutes and then the supernatant fluid was drawn off and further diluted to the point where 1 cc. of the fluid represented 5 to 10 mg. of the fresh anterior-lobe tissue. Varying amounts of this fluid were injected into the marginal ear vein of pregnant rabbits. In these experiments relatively large amounts of anterior-lobe substance were given, i.e., 25 to 100 mg. We have as yet made no effort to study ovulation in pregnant animals from a quantitative viewpoint. In two instances we induced ovulation in pregnant rabbits by intravenous injection of urine of pregnancy. In one experiment, ovulation was induced by subcutaneous injection of an anterior lobe from a non-pregnant sow, which was ground in saline and injected over a period of several hours.

In all experiments the ovaries and tubes have been fixed in Bouin's fluid for histological study. The tissue was stained

in Delafield's hematoxylin and eosin. A study of serial sections of the ovaries and fallopian tubes has convinced us that ovulation had occurred in every case.

EXPERIMENTAL

This report is based on data from ten pregnant rabbits in which ovulation was induced in various stages of pregnancy. In five of these animals the period of pregnancy was definitely known, while the other animals were pregnant when they were brought into the laboratory.

Ovulation was induced by the intravenous injection of fresh anterior-lobe substance as early as the fifth day and as late as the twenty-sixth day of pregnancy. Seven of the experimental animals were killed within thirty hours after the injection. Freshly ruptured follicles were observed in the ovaries in every case. Living fetuses and normal placentae were found in the uteri.

In two experiments the pregnant rabbits received intravenous injections of anterior-lobe substance on the fifth and the sixteenth days of pregnancy, respectively. One ovary and tube were removed from each animal on the day following the injection. Ovulation had occurred in both instances. The first animal was killed thirteen days after the injection. Nine living fetuses and normal placentae were observed in the uterus. The second animal was killed ten days after the injection. Living fetuses with normal placentae were found in the uterus.

In another experiment a rabbit in an early stage of pregnancy received an intravenous injection of anterior-lobe tissue. One ovary and tube were removed on the following day. Ovulation had taken place. Ten days later the animal received another intravenous injection of anterior-lobe tissue. The animal was killed on the following day. Seven fresh ovulations were observed in the remaining ovary, while living fetuses and normal placentae were found in the uterus.

DISCUSSION

Recently, Swezy and Evans ('30) have reported that at four- or five-day intervals in the pregnant rat, there is a wave of follicular growth, similar to that found in the non-pregnant animal. Moreover, newly formed corpora lutea were found, but in no instance were tubal ova observed. Loeb ('11) has described waves of follicular growth in the pregnant guinea-pig, but the newly formed follicles became atretic, ovulation never occurring.

It has been reported by Charipper and Haterius ('30) that there is an increase in the number of basophilic cells present in the anterior hypophysis of the non-pregnant female rat at the oestrous period. The observations of Swezy and Evans and of Loeb would indicate that there might be a rhythmic variation in the cell types present in the anterior lobe at different stages of pregnancy.

Nelson ('30) has recently reported an instance of an albino rat, which, although pregnant, exhibited the typical oestrous cycle of the non-pregnant animal. This animal delivered a normal litter. However, it was not ascertained whether ovulation had occurred during the oestrous periods. It has been clearly demonstrated by Hammond and Marshall ('25) that ovulation does not follow coitus in the pregnant rabbit. However, ovulation does occur after coitus within twenty-four hours after parturition. The investigations referred to above show that, although there probably are waves of follicular growth during pregnancy, this growth progresses only to a certain point and at this point atresia or luteinization sets in. The occurrence of oestrus and ovulation is rare.

The inhibition of oestrous changes and ovulation during pregnancy have been ascribed to the presence of the corpora lutea. It has been demonstrated by Hammond ('27) that the removal of the corpora lutea during the interval period in the cow will hasten the appearance of the next oestrus. Loeb ('11) has found the same to be true in the guinea-pig. Several investigators have shown that injections of extracts of corpora lutea into normal non-pregnant rats will inhibit

the appearance of oestrus (Parkes and Bellerby, '27; Papanicolaou, '26; Patel, '30). It has been assumed that the presence of corpora lutea prevents the appearance of oestrus and inhibits ovulation in the normal animal, but the mechanism of this inhibition is largely unknown. However, experiments in which ovulation has been produced in pregnant animals leads us to a better understanding of this problem.

It seems clear that the difference in the results as obtained by Zondek and Aschheim and by Engle and Mermod was due to the number of implantations of the anterior-lobe tissue given to the experimental animals. Engle and Mermod administered a series of daily implantations. At autopsy they found in the ovaries of the injected animals many large and medium-sized follicles. Recent corpora and many large cysts were also found. As many as twenty-nine ova were found in the fallopian tubes. With such an extensive follicular development, it must be assumed that there was an excessive production of oestrin. As pointed out by these investigators, the oestrin was probably the effective agent in terminating the pregnancy. It has been demonstrated by several investigators that injections of oestrin in the early part of pregnancy will prevent implantation and that large amounts in the later stages of pregnancy lead to resorption or abortion.

On the other hand, Zondek and Aschheim gave their experimental animals only one implant. The ovaries of their animals evidently did not undergo as great an amount of follicular development as did those of Engle and Mermod. The greatest number of ova found in any one tube was five. Also, there were very few large unruptured follicles in the ovaries. They described a beginning of cornification in the vagina of the pregnant mice, but these oestrous changes were not complete. It seems unlikely that there was the great production of oestrin in these animals as was the case in the animals of Engle and Mermod. Thus the pregnancy was not terminated.

In our experiments, we have used a single intravenous injection of fresh anterior-lobe tissue or of urine of pregnancy. It is doubtful that these injections caused any follicular development, but only caused rupture of the ripe follicles which were already present. It is known that many ripe follicles normally occur in the ovaries of pregnant rabbits (Hammond and Marshall, '25). In some instances only one or two ruptured follicles were found in each ovary, while in others as many as seven were found.

It must therefore be assumed that the follicular apparatus of pregnant animals can respond to anterior-pituitary stimulation. Moreover, it would seem that they have at least in some cases progressed far in their development and need only final stimulation to cause their rupture; if this stimulation is supplied in the form of anterior-lobe tissue, ovulation takes place. Patel ('30) has recently presented evidence to show that the simultaneous injection of an extract of the corpus luteum reduced the ovarian reactivity of immature mice to pituitary stimulation. However, no careful study of the comparative response of the ovaries of pregnant and non-pregnant animals has yet been made.

It seems highly probable that some factor other than the corpus luteum plays a part in the prevention of oestrus and ovulation in pregnant animals. It has been shown by Bacon ('30) and Philipp ('30) that the anterior pituitary of pregnant animals contains a reduced amount of the oestrogenic² hormone. Whether the corpus-luteum hormone may also play a part in the reduction of the oestrogenic capacity of the anterior hypophysis is at present an unanswered question.

SUMMARY

Ovulation has been induced in pregnant rabbits by: 1) A single intravenous injection of the urine of pregnancy; 2) a single intravenous injection of a saline suspension of fresh anterior-lobe tissue; 3) subcutaneous injection of anterior-

² The term oestrogenic hormone is used in this paper for the hormone which is produced in the anterior lobe of the hypophysis and which stimulates the development of the graafian follicles. See Wiesner and Crew ('30).

lobe tissue ground in sterile saline and injected over a period of five hours.

In one group of experiments the animals were killed within thirty hours after the injection. Ovulation had occurred in every instance. Normal placentae and living fetuses were found. In one rabbit ovulation was induced twice during the course of gestation with no injury to either the fetuses or placentae. Ovulation was induced in two rabbits early in the course of pregnancy. Two weeks later, normal placentae and fetuses were found in the uteri.

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THE CHANGES OCCURRING IN THE OVARY OF THE MARE DURING PREGNANCY

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FOUR TEXT FIGURES AND ONE PLATE (THREE FIGURES)

It has been shown in previous publications that sex hormones are present in the blood serum of mares during well-defined periods of pregnancy (Cole and Hart, '30, '30 a). An ovary-stimulating hormone (or hormones) is present as early as the thirty-seventh day and uniformly present by the forty-second day following fertilization. This hormone is present in the circulating blood until about the 150th day of pregnancy. We have no experimental data which would give evidence regarding the source of this ovary-stimulating principle. We have already drawn attention to the similarity of the ovarian reaction in the rats injected with the serum to that obtained by Smith and Engle ('27) with hypophyseal implants. However, the reactions are not identical. We have found that ovulation following injection of blood serum of mares is comparatively rare.

After about the 150th day the serum has an inhibiting effect upon the ovaries. It still has a stimulating effect upon the uterus and vagina, due no doubt to the presence of oestrin.¹

¹ The presence of these hormones makes it possible to diagnose pregnancy accurately from the fortieth day until approximately the end of the gestation period. Zondek ('30) advocated a method of diagnosing pregnancy in the mare by determining the concentration of oestrin and the follicle-stimulating hormone in the urine. We have found his method more tedious than that proposed by us with the use of serum. Recently the urine of a mare 264 days pregnant was tested with and without the treatment proposed by Zondek. We were surprised to find the urine highly reactive even though untreated. In the untreated urine 8000

The existence of these sex hormones in the blood caused us to become interested in the effect they might have on the ovaries of the pregnant mare. There has been no study made of the ovarian changes throughout pregnancy in any species in which the ovarian-stimulating substances have been found in the circulating blood. Kupfer ('28) studied the ovarian changes in the pregnant donkey mare, but there has been no study made of the hormones in this species. He states there is a striking similarity between the ovarian changes in this species and those in the mare. However, there is very little similarity between his findings in the donkey mare and our own in the mare. We agree with his findings at the end of pregnancy where he reports the absence of both corpora lutea and large follicles.

Swezy and Evans ('30) have reported in the rat that the cycle of ovogenesis is not interrupted by pregnancy, but is continued in much the same manner as in the ovaries of non-pregnant animals. Further, they report the occurrence of ripe follicles at the end of each period which ordinarily do not rupture, but proceed to the formation of corpora about a third the size of the corpora of pregnancy. Hammond ('27) found in the cow that follicles attained their full size during pregnancy with the exception of their preovulation increase. Cole ('30) confirmed this and also found differences in the character of the vaginal mucosa which he believed might be associated with periodic follicular growth and atresia.

MATERIAL AND METHODS

The material used in this study of thirty-five mares was obtained from an abattoir in which horses from the range

rat units of oestrin per liter were demonstrated, while approximately twice as much was found in the treated sample. No ovarian reaction was obtained in any of the experimental animals. In several cases tested in early pregnancy by using untreated urine unsatisfactory results were obtained, such as loss of the experimental animals and the fact that five of seven samples were completely negative while the serum from all seven animals gave definitely positive reactions. Data are lacking on the period of pregnancy when this test is reliable, as Zondek has only covered the period from the seventy-fourth to the 260th day. The comparison of the efficiency of the two methods indicates that the use of the serum in place of the urine gives greater opportunity for accuracy.

were slaughtered. The plant is located a few hours' distance from the laboratory by automobile. A total of eighty-one mares was examined postmortem, of which thirty-five were found to be pregnant. No breeding dates on these mares were available. A sample of blood, the fetus, and both ovaries were saved from each individual. Crown-rump and eye-ear measurements were made on the fetuses and the smaller ones were preserved in 10 per cent formalin. The ovaries were preserved in either Bouin's fixative fluid or 10 per cent formalin. After a short period of fixation, the ovaries were sliced with a razor blade into sections of about 0.4-cm. width. The slices were not completely severed from each other, thus allowing one to study the macroscopic appearance of the ovary at a later date.

The blood upon drawing was cooled in a refrigerator. The reaction of the serum upon the ovaries of immature rats was then determined by injecting from three to six twenty-one-day-old female rats with the serum.

EXPERIMENTAL

Our observations on these mares show that in this species the cyclic ovarian changes associated with the recurring oestrous cycles of eighteen to twenty-one days' duration do not occur during pregnancy. The more or less prolonged changes which do occur are none the less striking and are distinctly correlated with the variations in the sex-hormone concentrations in the blood serum of the animal.

The entire pregnancy interval may be divided into four periods as regards the manifestation of definite changes in the ovary.

Period 1

The first period is quite definite as to time, extending from conception until slightly after the fortieth day. The time can be definitely placed in the absence of breeding dates by the reactions to the serum. The earliest positive reaction is with serum from mare 31, the fetus of which had a crown-rump measurement of 1.9 cm. (table 1). It will be observed that

TABLE 1

Data on the ovaries of pregnant mares sacrificed at various periods of pregnancy together with the crown-rump length of their fetuses, and the reactions of the injected blood sera of the mares upon the genitalia of immature rats. Note: Blood samples from mares 77, 80, 81, and 11 were not tested

DESIGNATION OF MARE	CROWN-RUMP LENGTH OF FETUS, CM.	REACTION OF INJECTED MARE SERUM UPON GENITALIA OF IMMATURE RATS			DATA ON MARE OVARIES		
		Ovary	Uterus	Vagina	Number of follicles with diameter of 1.0 cm. or more	Number of forming corpora lutea	Number of solid corpora lutea
40th to 150th day of pregnancy, approximately	19	—	—	—	6	None	1
	27	—	—	—	3	None	1
	15	—	—	—	7	None	1
	23	—	—	—	None	None	1
	22	—	—	—	5	None	1
	50	—	—	—	4	None	1
	31	+	++	+	2	None	1
	38	++	++	+	3	1	1
	43	++	++	+	6	None	1
	37	++	++	+	4	1	1
	28	++	++	+	4	1	1
	45	++	++	+	7	None	2
	29	++	++	+	3	4	1
	35	++	++	+	None	2	5
	48	+	++	+	None	None	1
	40	++	++	+	8	None	2
	30	++	++	+	4	10	1
	42	++	++	+	6	7	2
	44	++	++	+	1	8	1
	53	++	++	+	None	11	1
	61	+	++	+	None	None	2
	54	+	++	+	None	None	11
	49	+	+	+	None	None	10
	60	—	+	+	None	None	2 vestiges
	77	—	—	—	None	None	None
	80	—	—	—	7	None	None
	9	—	+	+	None	None	None
	81	—	—	—	None	None	1 vestige
	12	—	+	+	None	None	None
	76	—	+	+	None	None	None
	11	—	—	—	None	None	None
	75	—	+	+	None	None	None
	78	—	+	+	2	None	None
	73	—	+	+	None	None	None
	74	—	+	+	None	None	None

mare 50, with a fetus also measuring 1.9 cm., was negative to the serum test. This is evidence that when the fetal crown-rump measurement is 1.9 cm., the duration of pregnancy has been about forty days. The end of period 1 is marked by the beginning of luteinization of follicles, as shown first in mare 38, carrying a fetus with a crown-rump measurement of 2.2 cm. The serum of mare 38 was highly positive.

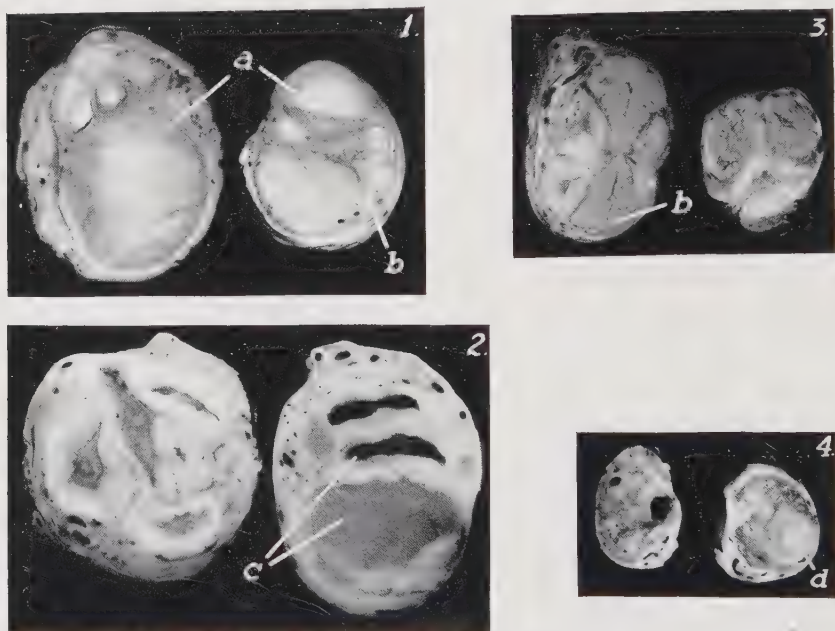
During this period there is one corpus luteum in one of the two ovaries which is the corpus luteum of pregnancy, and follicles of variable size in each of the two ovaries (fig. 1). Our series does not include any cases of pregnancy much shorter than thirty days. This may explain why the corpus luteum of heat periods previous to the one at which conception took place were not present. Some vestiges were found. This is evidence, which will be added to later, that the corpus luteum in this species may involute rapidly.

Although the ovaries contain large follicles in this period, the blood serum has no effect upon the ovaries of immature rats in doses as great as 60 cc. This is analogous to the condition in the non-pregnant animal, where ripe follicles are being produced periodically with no evidence of ovary-stimulating substances in the blood. The exception to this, as shown in table 1, is in the latest part of this period, when the blood serum becomes positive. The data suggest that the concentration of the hormone may result in the luteinization phenomenon. Table 1 gives the number of follicles 1 cm. or more in size. There were, however, follicles included in the table as large as 7 cm. in diameter, and mare 23, listed with none, did have two follicles 0.7 cm. in diameter. The average size of these follicles was about 2 cm. and it was rare to find any larger than 3 cm.

Period 2

In this period, extending from about the fortieth to the 150th day of pregnancy, there is a progressive activity in the ovary. This is manifested by continued formation of follicles and marked luteinization. The luteinization of the follicles

beginning in the early part of this period is manifested by one or two follicles showing this change. Mares nos. 40, 30, 42, 44, and 53, killed late in this period, show from ten to fifteen follicles over 1 cm., forming corpora lutea, or solid corpora lutea (fig. 2). This activity in the ovary may be due



Figures 1 to 4 are photographs of unstained cross-sections of both ovaries of four mares sacrificed at different periods of pregnancy. *a*, follicles; *b*, solid corpora lutea; *c*, forming corpora lutea; *d*, regressing corpora lutea.

Fig. 1 From mare 19, pregnant less than forty days (crown-rump length of fetus, 1.2 cm.). Note the corpus luteum of pregnancy and the follicles of varying size. $\times \frac{1}{2}$.

Fig. 2 From mare 53, pregnant about 120 days (crown-rump length of fetus, 22.0 cm.). Seven corpora lutea may be seen in the two ovaries in different stages of development. $\times \frac{1}{2}$.

Fig. 3 From mare 54, pregnant about 140 days (crown-rump length of fetus, 26.3 cm.). The ovaries are filled with solid corpora lutea in the first stages of regression. No large follicles are present. $\times \frac{1}{2}$.

Fig. 4 From mare 60, pregnant about eight months (crown-rump length of fetus, 43.0 cm.). Only vestiges of corpora are present. Ovaries in later stages of pregnancy resemble these, with the exception that the corpora-lutea vestiges are more minute. $\times \frac{1}{2}$.

to the cumulative effect of the ovary-stimulating hormone existing in the blood at its highest concentration for some time. The presence of the ovary-stimulating hormone may be necessary to maintain the functional activity of the corpus luteum of pregnancy. The additional corpora lutea produced at this time may be a factor of safety in insuring implantation. In a previous publication Cole and Hart ('30) have drawn attention to the correlation between the time of high concentration of the ovary-stimulating hormone and implantation. The luteinization phenomenon occurring during this period may explain why this correlation exists. Possibly the functional activity of the corpus luteum is maintained by other means in species in which it is impossible to demonstrate the ovary-stimulating hormone in the blood during pregnancy.

The query whether or not the luteinization of the follicle is preceded by ovulation is logical. Inasmuch as the rupture point resulting in the corpus luteum of pregnancy is absent or indistinct in the late part of period 1, it would indicate that the corpora do not remain visible for any considerable length of time and they tend to recede instead of project from the surface of the ovary. In eight out of thirteen cases during period 2 distinct rupture points were visible. In one mare four rupture points were found in the two ovaries (mare 35). It is therefore logical to conclude that ovulation quite frequently occurs.

The histological study of these corpora is interesting. The youngest corpora are usually lace-like structures, differing from those figured by Evans and Cole ('31) in the dog in only one important detail. This is in the fact that the antrum is almost invariably filled with red blood cells and blood pigment. One ovary from mare 38 contained a young corpus luteum in which there was extravasation of red blood cells at the periphery rather than into the antrum. Instances in which one or two layers of lutein cells were separated from the antrum by a connective-tissue layer were also encountered. The more fully developed corpora may be compared to the

corpora hemorrhagica described by Aschheim and Zondek ('28) in the ovaries of immature mice injected with urine from pregnant women. The blood cells and pigment remain in the antrum until it is obliterated by the ingrowing lutein tissue (fig. 2). The ingrowth of connective tissue and blood vessels often proceeds faster than the lutein cells, thus lining the circumference of the antrum with a connective-tissue border. It is impossible to distinguish these corpora when fully developed from the corpus luteum of pregnancy (fig. 6). We are not able to find any corpus, including the corpus luteum of pregnancy, which is undergoing any regression until the end of this period.

Period 3

The distinguishing characteristic of this period is the regression of the corpora lutea. Large follicles are completely absent during this time (fig. 3). This period begins about the end of the fifth month. We do not have sufficient material and it would be difficult to accurately determine its close. Mare 60, carrying a fetus with a crown-rump measurement of 43 cm., had only vestiges of corpora present, from which it might be concluded that the period terminates when approximately two-thirds of the pregnancy interval has elapsed. Figure 4 shows cross-sections of both ovaries of mare 60 in which the corpora have undergone practically complete regression. Figure 7 shows the microscopic appearance of this corpus luteum atretica. There is a marked decrease in size of the lutein cells, together with their vacuolation. Fat stains show these vacuoles to contain large fat globules. We particularly desire to call attention to the correspondence of time between the beginning of regression of the corpora with the disappearance of the ovary-stimulating substance from the blood stream.

Period 4

In those species studied it has been found that the corpus luteum of pregnancy persists to the end of gestation. In the

mare we find the ovary at the end of pregnancy to be entirely lacking in corpora lutea. Minute vestiges of old corpora may still be seen in most instances. It is interesting to note that, although large follicles were found in the ovaries of only two of eleven mares, nos. 78 and 80, oestrin is nevertheless regularly present in the blood stream in considerable concentration.

SUMMARY

Ovaries, fetuses, and blood samples were procured from thirty-five range mares throughout the period of pregnancy. The duration of pregnancy was determined by the reaction of the injected blood serum upon the ovaries of immature rats and by measurement of the fetuses.

The outstanding result of the study was the fact that we were able to divide the gestation interval into four periods, depending upon the condition of the ovaries. Each of these periods may be characterized as follows:

Period 1. A single corpus luteum of pregnancy and several follicles. Interval, about first forty days of pregnancy.

Period 2. Time of formation of several corpora lutea and closely correlated with the time of high concentration of the ovary-stimulating hormone in the blood. Ovulation apparently occurs in some instances during this period. Interval, fortieth to 150th day of pregnancy.

Period 3. Regression of all of the corpora lutea and absence of large follicles. Interval, from about the 150th day to late stages of pregnancy.

Period 4. Only minute vestiges of corpora lutea and rarely follicles larger than 1 cm. in diameter. Interval, late stages of pregnancy.

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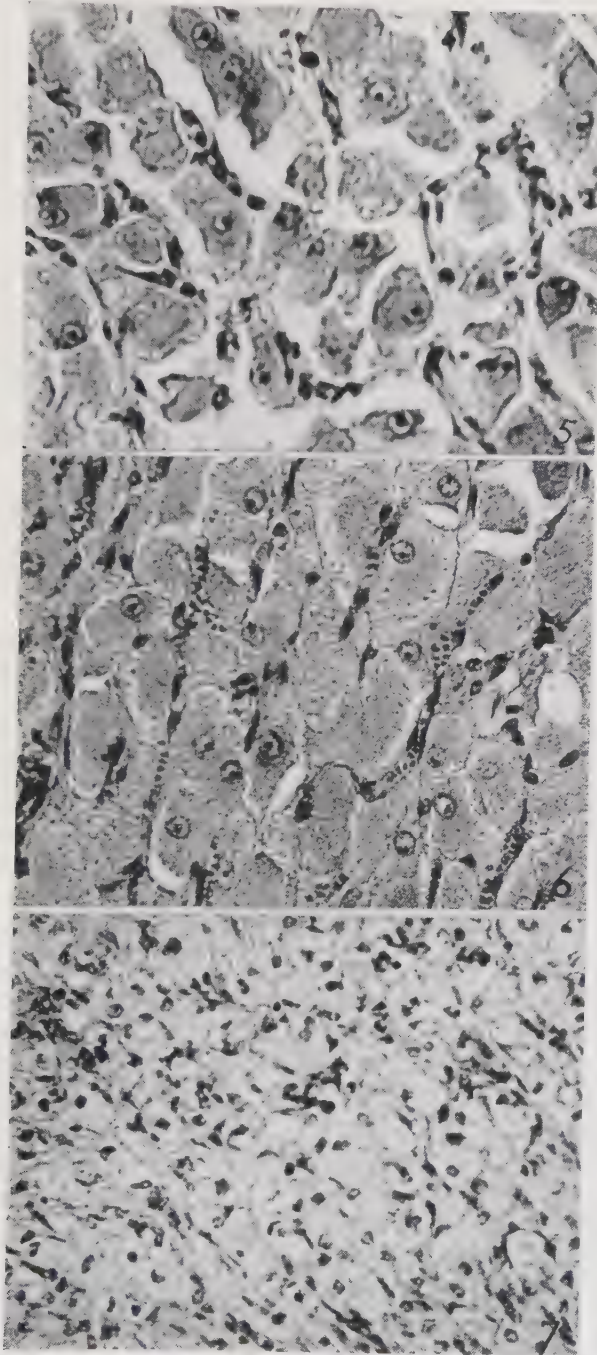
PLATE 1

EXPLANATION OF FIGURES

5 Photomicrograph of a section of a developing corpus luteum from mare 38 (crown-rump length of fetus, 2.2 cm.). Another fully developed corpus luteum, the corpus luteum of pregnancy, similar to the one illustrated in figure 6 is present in the same ovary. $\times 336$.

6 Photomicrograph of a fully developed corpus luteum of pregnancy from mare 23 (crown-rump length of fetus, 1.8 cm.), showing a compact mass of functioning lutein cells. $\times 336$.

7 Photomicrograph of a regressing corpus luteum from mare 60 (crown-rump length of fetus, 43.0 cm.). $\times 336$.



A DEMONSTRATION OF EQUIVALENT POTENCIES OF RIGHT AND LEFT TESTIS-LIKE GONADS IN THE OVARIOTOMIZED FOWL¹

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ONE DIAGRAM AND THREE PLATES (SIX FIGURES)

INTRODUCTION

The effects of sinistral ovariectomy in the fowl have been studied by several investigators in recent years. In most of these investigations special emphasis was placed on the hypertrophy of the rudimentary right gonad into an organ usually of testis-like form and structure, though almost invariably sterile. In a few of these cases—Benoit ('23), two, and Zawadowsky ('26), one—spermatogenesis was found in such right gonads. The writer has recently confirmed these observations, having found twelve new cases, and explained the reason for their occurrence (Domm, '28 a and '29 b). By none of these investigators has special emphasis been placed on the occasional development of a left testis-like gonad on the site of the removed left ovary. A convincing demonstration of equivalent potencies of these testis-like gonads on right and left sides of the sinistrally ovariectomized fowl has not been given. It is the purpose of the present paper to present definite evidence that the left testis-like gonad frequently found in sinistrally ovariectomized fowl has endocrine potentialities equivalent to the right compensatory testis-like organ.

¹ This investigation was supported in part by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

The removal of the normal left ovary in the Leghorn brings about a general masculinization of sexual characters, so that the female ultimately resembles the male more or less completely in external characterization. Plumage and spurs masculinize in the absence of ovarian tissue in a very brief period. This is particularly striking with reference to plumage, for the actively growing feather reveals the change from female to male following sinistral ovariectomy in the course of a few days and by two weeks after the operation new male plumage may be quite noticeable. The rudimentary spurs of the hen likewise reveal growth soon following sinistral ovariectomy. There is usually a reduction in size of head furnishings following the operation excepting in instances where these characters are small, as in the chick. The head furnishings will again manifest signs of growth at varying periods following the operation and may ultimately attain masculine dimensions. Whenever such individuals show masculine head apparel, they almost invariably reveal some masculine behavior. The latter seem closely associated characters and are conditioned by the hypertrophied testis-like rudimentary right gonad. Here we propose to demonstrate that the left testis-like gonad, frequently developed on the site of the removed left ovary (Domm, '24, '27, '28 a, '29 b), alone may bring about equivalent modifications.

The inherent difficulties involved in making such a demonstration are considerable and this accounts no doubt for the fact that conclusive observations of this sort have not previously been made. One of the invariable sequelae of sinistral ovariectomy in our experiments has been the hypertrophy of the rudimentary right gonad. Our experiments reveal this to be an inevitable sequence so conclusive that it needs no further emphasis. The type of gonad ultimately developed, however, varies in individual cases, depending apparently upon the embryological rudiments that persist. Hence the gonad found may be either testis, ovary, or ovotestis, the latter representing a combination of the former two in varying proportions.

Concerning the left gonad site, the possibilities for gonad development, following sinistral ovariectomy, depend on a different set of conditions and the probabilities of getting a testis-like gonad are proportionately much less than on the right site. If the operation has been absolutely complete, no regeneration follows; or, on the contrary, if it has been very incomplete, ovarian tissue will rapidly regenerate, inhibiting development of testis-like gonad on either site. In cases where small masses of cortical and medullary tissue remain following the operation, an ovotestis may result. In cases where testis alone develops, it is assumed that all cortical tissue had been removed, while some medullary tissue remained which hypertrophied into a testis-like gonad.

In order to demonstrate the inherent capacities of such left testis-like growths, it will thus be necessary to select ovariectomized fowl showing such growths, determined by laparotomy, and, by a second operation, remove the hypertrophied gonad on the right side. This secondary operation is fraught with difficulties even more severe than the initial sinistral ovariectomy, so that the chances for success are small even where one has acquired considerable dexterity. On the other hand, one may perform an initial dextral ovariectomy in which the rudimentary right gonad is destroyed by cautery (Domm, '29). This method obviates the difficulties inherent in removing a hypertrophied right gonad. One may then subsequently remove the left ovary and chance the development of a testis-like gonad in its stead. Or the latter method may be slightly varied to the extent of first performing a sinistral ovariectomy, followed immediately upon recovery, before the right gonad has hypertrophied, by a second dextral ovariectomy, destroying the right rudimentary gonad by cautery as before. This method obviates some of the technical difficulties, as related above, though it is also not altogether satisfactory, since one has no way of definitely controlling the type of gonad, if any, that will develop on the left.

The cases constituting the present series were not produced by an intentional decortication of the ovary, as the preceding

description would imply. Each of the six cases included in the series belonged to another series (Domm, '29 a) on which bilateral ovariectomies were performed with the intention of producing gonadless birds. Hence in each instance the bird had an initial sinistral ovariectomy followed soon upon recovery, in these cases from sixteen to twenty-two days (see table), by a second dextral ovariectomy in which the right rudimentary gonad was destroyed by electric cautery. Those birds that subsequently showed signs of gonad activity, as disclosed principally by comb growth, were given a careful laparotomy to determine the presence of traces of right gonad. None of them revealed right gonad at this time, though all suspicious tissue was carefully removed and the site again thoroughly seared to preclude subsequent regeneration. The left gonads in each instance were left intact. Of the cases thus treated, six survived and lived a sufficient period to satisfactorily indicate the effects of the left testis-like gonad. Each case upon autopsy revealed a relatively prominent testis-like left gonad, comparable to that usually found on the right, while in none was there any indication of gonadal tissue on the right. A general categorial description of the physiological effects of these left testis-like organs will be given. The essential features of the various cases will be given in the text with not infrequent references to the table, which gives detailed case histories to which the reader is referred for supplementary data. Details on methods of operation, records, and preservation of materials were related in previous papers (Domm, '27 and '29 a, b).

This investigation is part of a larger program on the biology of sex now being pursued at this laboratory under the direction of Prof. Frank R. Lillie. It is a pleasure to acknowledge the assistance of Professor Lillie in making these studies possible.

REGENERATION OF LEFT TESTIS-LIKE GONADS

The development of left testis-like gonads following sinistral ovariectomy, in conjunction with right testis-like gonads, has been previously observed in a few cases by several investigators, though not diagnosed as such by all. Goodale ('16) evidently had such a case which revealed on the site of the removed ovary 'a mass of whitish tissue.' Benoit ('23) found a similar growth on the left gonad site in a bird ovariectomized when four days old. Zawadowsky ('22 and '26) records two cases in which a left testis-like gonad developed following sinistral ovariectomy similar to that usually encountered on the right. In none of these cases was any attempt made to remove the hypertrophied right gonad in order to study the potentialities of these left testis-like gonads. Zawadowsky ('26), however, has intimated, but not demonstrated, equivalent potentialities of right and left testis-like gonads, for he says (p. 170):

My experiments prove that the right sexual gland develops only upon removal of the left ovary. However, in the course of my experiments I fell upon one case when the right sexual gland of a castrated hen developed although the left sexual gland was regenerating. But it is characteristic that the left sexual gland of this hen had developed after the type of a right sexual gland and not after the type of a normal left ovary.

Footnote, same page:

Hen 'Aphrodita' was not the only one of my experimental hens which was inclined to form seminiferous tubules in its left regenerating ovary. In my study . . . 1922, (pages 82-84) I mentioned the hen 'metiss.' The regeneration process of its left gland resulted in the growth of a cock's comb. Hence already then I made the following conclusion: "the left ovary is also potentially capable of secreting maskulinisin elements."

However, in reading the history of this case we find no reference to the removal of the right gonad; in fact, no right gonad is mentioned, though his own later statement (see reference above), as well as our own observations, leads to the supposition that the bird mentioned had a right gonad, since

the right rudiment invariably hypertrophies following ovariectomy. Perhaps the right gonad in this case was relatively small and consequently regarded as insignificant.

In our own work (compare Domm, '24, '27, '28 a, and '29 b) many such cases of left testis-like gonads have been encountered in sinistrally ovariectomized fowl. In one of these papers (Domm, '27) forty-two cases of left testis-like gonads were found out of a total of 121 sinistrally ovariectomized fowl. Additional cases have since been found. In all of these cases a right gonad of varying proportions was always associated. Hence in none of these cases could we logically assign the development of dependent masculine sex characters only to the influence of the left testis-like gonad, though it obviously may have been contributory. We did, however, earlier demonstrate, though not as completely as we should have liked, the equivalent potentialities of right and left testis-like gonads. In this case, after removal of the right compensatory gonad, the left testis-like gonad supported the dependent masculine characters for a period of one month, when it was also removed, following which the bird became capon-like for a period of approximately four months (compare Domm, '27, p. 57 et seq.).

Macroscopically the left testis-like gonad found in the fowl is similar to the testis-like right gonad; detailed descriptions of both were previously given (Domm, '27). The size of the gonad, probably controlled to some extent by the amount of medullary tissue remaining following the operation, is, on the average, somewhat below that on the right, though there are cases in which it is approximately of similar size or even larger (compare figs. 1164, 892, 881, and 885, pls. 2 and 3).

EFFECT OF ACCESSORY ORGANS OF REPRODUCTION

Castration brings about definite changes in the reproductive ducts of both sexes. The prominent convoluted vasa deferentia of the male are greatly reduced and lose their convolutions following castration. The wolffian ducts in the female are the homologues of the vasa deferentia. These per-

sist as small slender threads in the normal female. Upon sinistral ovariectomy, they hypertrophy and frequently become convoluted under the stimulus of the hypertrophying rudimentary right gonad. Subsequent excision of hypertrophied gonads causes retrogression of ducts, whereas complete early bilateral ovariectomy is ineffective, the ducts retaining their rudimentary condition.

The birds of this series with only left testis-like gonads revealed modifications of the accessory organs equivalent to those bearing only right testis-like gonads (compare figs. 1164 and 881, 885, 892, pls. 2 and 3). In each of the six cases the wolffian ducts revealed readily demonstrable growth. In birds nos. 873, 881, 883, 885, and 889 these ducts had increased in size and in each case were slightly convoluted. The convolutions in most of these are confined to the posterior ends (figs. 881 and 885, pl. 3), this region of the duct probably being the first to undergo this modification. In each of the five cases they had attained approximately the same size (see table). Bird no. 892 revealed the most extensively hypertrophied wolffian ducts. These had a conspicuous diameter and were strongly convoluted throughout their entire length (see table, also pl. 2). In all cases right and left vasa deferentia were equivalent in size. The right is always readily demonstrable, whereas the left is more difficult to find, owing to accumulations of fat in the mesentery of the oviduct wherein the vas lies. In none of our ovariectomized birds have we ever witnessed vasa deferentia equivalent in size to those of the normal male. This was somewhat baffling at first, but when it was found that the vas in a unilaterally castrated male was smaller on the side of the removed testis than on the side of the intact testis, most of the difficulty disappeared, since such vasa are approximately the same size as the largest frequently found in sinistrally ovariectomized fowl. The larger size of the vas on the side of the intact testis in the unilateral castrate is evidently due to the fact that it is distended with spermatozoa and secreted fluids.

Following complete bilateral ovariectomy, or in the complete absence of all gonad, the oviduct of the female becomes reduced to a small, straight, flattened tube having a diameter of but 2 to 3 mm. (Domm, '29 a). This represents a tremendous reduction when one considers the large glandular oviduct of a laying hen. In comparison the reduction of the much smaller normal vas deferens following castration is relatively insignificant. Following complete sinistral ovariectomy, there is also a marked reduction in size, followed by varying subsequent increases in different cases up to approximately normal size in some. We have previously called attention to these modifications and also emphasized the fact that there is a correlation between plumage reversion and stimulation of oviduct indicating presence of female hormone (compare Domm, '27, '28 a, and '29 a).

The oviducts in the birds of this series showed no marked deviation from those observed in sinistrally ovariectomized

EXPLANATION OF TABLE

The table gives the history of those cases described in the text which developed left testis-like gonads following bilateral ovariectomy as determined by laparotomy and postmortem examination. On account of the length of the record, each case is continued on a second page. The record of each case consists of selections from very much more complete records considered to be most important for the operation history. In some cases other data are recorded in the text. The preserved records consist of notebooks containing complete histories of all birds, photographs, feather records for each case, skins, preserved sacra with urogenital organs in situ for each case, and other anatomical preparations.

Column 1 gives the identification number of each bird. Column 2 gives the age of the bird in days at the time of the first sinistral operation. It also gives the dates of the sinistral and dextral operations in their order. The sinistral operation always preceded. Column 3 gives the date on which the bird was killed and autopsied.

'Successive changes in plumage' and 'successive changes in head furnishings' are recorded at 3, 6, 12, 18, 25, and 30 months following the date of operation. Measurements of head furnishings at the time of the initial sinistral operation are also recorded. These periods are not always the best for recording changes, hence observations at other times are frequently entered in the nearest column and indicated by a number in parenthesis giving the actual age in months above the individual entry. All such dates given in months are approximate only, though they are rarely more than a few days off, but on account of the relative slowness of the changes they are sufficiently exact.

Plumage changes. The changes recorded are, in general, the natural plumage changes, not forced by extensive plucking. The only exceptions are operation sites, though, because of the rapid development of male plumage in most of these cases immediately following operations, these are not long apparent. In several cases, aside from periodic plucking for record purposes, small areas were plucked from different regions in order to better determine their differential thresholds. ♂ indicates feathers of cock or capon type not distinguished. ♀ indicates feathers of female type.

'Tipped' always refers to feathers with female tip and male base which appear shortly after ovariectomy; these feathers commonly have a very sharp line of demarcation between the components, and are frequently referred to as 'gynandromorph' feathers in the literature. Such feathers may be noticed by ten to fourteen days after the operation and were abundant at three months, interspersed with new completely male feathers.

I signifies 'intermediate,' and here represents the beginning of the secondary transformation from the male to the female type, or a reversion back to the male type of feathers. These feathers may have male tips and female bases, but the transition zone is not sharp but diffuse. In some instances the entire feather is intermediate or of this diffuse nature.

Where regions are indicated, abbreviations are used, viz.: *Br.* for breast, *Ba.* for back, *Sa.* for saddle, *T.* for tail, *W's.* for wings, *Wc's* for wing coverts, *Juv.* for juvenile, etc.

Head furnishings (measurements are in centimeters). The comb is given first, the length of the main blade of the comb from front to back being the numerator and the greatest depth from the highest point to the base the denominator; the wattles come second, width over depth; the vertical diameter of the ear lobe comes last.

Spurs. Spurs are recorded by their length in centimeters at date of autopsy.

Findings at autopsy. At the time of autopsy, the head was preserved separately in formalin, the skin with legs attached removed and cured, and the entire sacrum with urinogenital organs, including gonads, fixed in Bouin's fluid.

Right and left gonads. *None* signifies no gonad. *T* signifies 'testis-like' gonad macroscopically. Measurements are length over transverse diameter, in centimeters.

Right and left *V.D.* (vas deferens). For purposes of succinct characterization the arbitrary scale previously devised (Domm, '27 a) was utilized in which 1 corresponds to the condition of the normal right vas deferens in the female and 5 that of the male; 2, 3, and 4 represent intermediate conditions: 2, wide, straight; 3, slightly convoluted; 4, strongly convoluted. Observations are more difficult to make on the left side, on account of accumulations of fat in the mesentery of the oviduct where the vas lies.

Right and left *Ovd.* (oviduct). Similarly, a scale of six points was adopted for recording variations of the left oviduct. 1, the most reduced type, straight and only 2 to 3 mm. in diameter; 2, straight, 4 mm. or more in diameter; 3, convoluted, 3 to 5 mm. in diameter; 4, convoluted, 6 to 9 mm. in diameter; 5, convoluted, 10+ mm. in greatest diameter; 6, oviduct of a normal laying hen (Domm, '27; compare pl. 8, fig. 2b; pls. 9 and 10; also pl. 11, no. 729). † Varying portions of oviduct inflated with fluid (see text, p. 222, also fig. 881, pl. 3).

* signifies remnant of right oviduct in form of cloacal stump.

Summary of bilaterally ovario

BIRD NO.	OPERATION AGE AND DATE	AUTOPSY DATE	SUCCESSIVE CHANGES IN PLUMAGE						Rt
			3 months	6 months	12 months	18 months	25 months	30 months	
873	76 d. 9-14-26 9-30-26	7-30-29	Predom. ♂. Some juv. Some old ♀ and ♀ tipped. New ♂.	♂ in all areas. Few scattered ♀ Wc's. Few scattered juv. New ♂.	♂ in all areas. Numerous new ♂. Molting.	♂ in all areas. New ♂.	♂ in all areas. New ♂. Molt complete.	(34½) ♂ in all areas. New ♂.	4.
881	78 d. 9-16-26 9-30-26	7-30-29	Predom. ♂. Many juv. on Ba. and Sa., few on W's. Some old ♀ and ♀ tipped. New ♂.	♂ in all areas. Some juv. Few scattered old ♀. New ♂.	♂ in all areas. New ♂. Molt complete	♂ in all areas. New ♂.	Predom. I. Few ♂ and ♀. New Br. I.	(34½) Predom. I. Many ♀. Few ♂. New ♀.	3.
883	78 d. 9-16-26 10-5-26	11-2-27	Predom. ♂. Many juv. on Ba., Sa., and W's. Few old ♀. New ♂.	Predom. ♂. Few scattered juv. and I. New Ba. and Sa. ♀. New Br. ♂.	Predom. ♀. Some ♂ and I. New ♀. Molting.	(13) Predom. ♀. Some ♂ and I. New Br. and W.I., Ba., and Sa. ♀.			1.
885	78 d. 9-16-26 10-6-26	4-22-29	Predom. ♂. Many juv. on Ba., Sa., and W's. Few old ♀ and ♀ tipped. New ♂.	♂ in all areas. Some juv. Few old ♀. New ♂.	♂ in all areas. New ♂. Molt complete.	♂ in all areas. New ♂.	Predom. ♀. Some ♂ and I. prin. on Ba. and Sa. New ♀. Molting.	(31½) ♀ in all areas except tail. New ♀.	3.
889	78 d. 9-16-26 10-6-26	7-30-29	Predom. ♂. Many juv. on Ba. and Sa., few on W's. Few old ♀ and ♀ tipped. New ♂.	♂ in all areas. Some scat- tered juv. on Ba., Sa., and Wc's. New ♂.	♂ in all areas. New ♂. Molt complete.	♂ in all areas. New ♂.	♂ in all areas. New ♂.	(34½) ♂ in all areas. New ♂.	3.
892	79 d. 9-17-26 10-5-26	7-30-29	Predom. ♂. Many juv. on Ba., Sa., and W's. Some old ♀ and ♀ tipped. New ♂.	Predom. ♂. Some old ♀ and ♀ tipped. New ♀.	Approx. ½ ♂, ½ ♀, some I, new ♀. Molting.	Predom. ♂. Many ♀. Some I. New ♀.	Predom. ♂. Many I. Fewer ♀. New Br. ♂. Molting.	(34½) Predom. ♂. Many I. Some ♀. New ♀.	4.

with left testis-like gonads

SUCCESSIVE CHANGES IN HEAD FURNISHINGS							FINDINGS AT AUTOPSY					
At operation	3 months	6 months	12 months	18 months	25 months	30 months	Rt. gonad	Lt. gonad	Rt. V.D.	Lt. V.D.	Rt. Ovd.	Lt. Ovd.
$\frac{2.2}{0.8} \frac{1.3}{0.3} 1.0$	$\frac{3.1}{1.6} \frac{1.6}{0.6} 1.3$	$\frac{4.4}{3.1} \frac{2.1}{1.3} 1.3$	$\frac{8.1}{5.1} \frac{2.7}{2.3} 1.9$	(19) $\frac{11.1}{7.9} \frac{4.2}{4.0} 2.1$	$\frac{9.6}{6.9} \frac{3.3}{3.2} 2.3$	(34½) $\frac{10.6}{7.6} \frac{3.3}{3.6} 2.4$	None	T $\frac{1.4}{0.6}$	3	3	*	1
$\frac{2.1}{0.5} \frac{1.5}{0.2} 1.0$	$\frac{2.7}{1.2} \frac{1.6}{0.5} 1.2$	$\frac{8.8}{3.9} \frac{2.9}{2.2} 1.7$	$\frac{7.1}{4.5} \frac{2.7}{2.6} 1.9$	$\frac{7.7}{5.3} \frac{2.9}{2.8} 1.8$	$\frac{7.6}{4.3} \frac{3.0}{2.9} 2.1$	(34½) $\frac{9.7}{6.0} \frac{3.5}{3.7} 2.3$	None	T $\frac{1.9}{1.0}$	3	3	*	4 †
$\frac{2.0}{0.9} \frac{1.3}{0.2} 1.2$	$\frac{2.7}{1.5} \frac{1.7}{0.4} 1.4$	$\frac{8.5}{5.2} \frac{3.8}{3.4} 2.1$	(9) $\frac{9.8}{5.7} \frac{4.0}{4.1} 2.3$	(12) $\frac{8.7}{4.8} \frac{3.4}{3.7} 2.4$	(13½) $\frac{7.7}{4.3} \frac{2.7}{2.5} 2.1$		None	T $\frac{2.0}{0.8}$	3	3	*	4
$\frac{1.8}{0.6} \frac{1.4}{0.3} 0.8$	$\frac{2.5}{1.0} \frac{1.4}{0.4} 1.3$	$\frac{3.9}{1.9} \frac{2.1}{1.3} 1.3$	$\frac{8.1}{4.6} \frac{3.4}{3.0} 2.3$	(19) $\frac{10.6}{6.0} \frac{4.2}{3.8} 2.3$	$\frac{6.5}{2.5} \frac{2.3}{2.6} 2.3$	(31½) $\frac{9.4}{4.5} \frac{3.4}{3.2} 2.3$	None	T $\frac{1.8}{0.7}$	3	3	*	3
$\frac{2.0}{0.5} \frac{1.6}{0.3} 1.0$	$\frac{2.8}{1.2} \frac{1.6}{0.3} 1.2$	$\frac{3.2}{1.5} \frac{2.0}{0.6} 1.7$	$\frac{4.6}{1.7} \frac{2.3}{1.6} 1.8$	$\frac{4.8}{1.6} \frac{2.4}{1.6} 1.8$	$\frac{8.0}{3.3} \frac{3.7}{3.1} 2.2$	(34½) $\frac{12.6}{6.4} \frac{5.4}{5.7} 2.9$	None	T $\frac{1.4}{0.5}$	3	3	*	1 †
$\frac{1.8}{0.8} \frac{1.3}{0.4} 0.9$	$\frac{2.8}{1.4} \frac{1.6}{0.5} 1.2$	(7) $\frac{11.0}{6.5} \frac{4.6}{4.6} 2.9$	$\frac{9.8}{5.8} \frac{4.0}{4.5} 2.8$	(19) $\frac{11.6}{7.0} \frac{4.7}{5.5} 3.3$	$\frac{9.7}{5.7} \frac{3.7}{4.5} 3.2$	(34½) $\frac{11.1}{6.6} \frac{4.9}{5.8} 3.5$	None	T $\frac{3.2}{1.4}$	4	4	*	3

birds. The most reduced oviducts, curiously enough, were found in birds nos. 873 and 889, neither of which, up to the time of killing, had shown any indications of plumage reversion, denoting, we believe, that the quantity of female hormone produced, if any, had not yet become very great. The gonads in each were approximately the same size and the smallest of the series. The oviducts of birds nos. 885 and 892 were about the same size, they were somewhat larger than the above, were slightly convoluted, and had a diameter of 3 to 5 mm. (see table, also pls. 2 and 3). Both oviducts were unquestionably stimulated by female hormone, which is also indicated by the fact that in both there was suppression of male plumage which was manifest relatively early in bird no. 892 (see table). The oviducts of birds nos. 881 and 883 were the largest of the series, and these were somewhat larger than the preceding, having a diameter of 6 to 7 mm. (pl. 3). Each of these cases also revealed reversion in plumage (see table). Parts of the oviduct of birds nos. 881 and 889 were distorted, owing to distention with fluid giving a false impression of their size (fig. 881, pl. 3).

EFFECT ON HEAD FURNISHINGS

The birds discussed in this report were young growing pullets at the time of the operation whose head furnishings were small and in a juvenile condition. As previously pointed out, castration in the female has little, if any, perceptible effect on head furnishings during this stage (Domm, '27). In cases where the operation is performed later, when head furnishings have revealed considerable growth, the immediate effects may be more pronounced, depending on size attained. In such instances there is usually considerable reduction, followed by subsequent growth at varying periods after sinistral ovariectomy (Domm, '27), while in complete bilateral ovariectomy no such growth ensues (Domm, '27 and '29 a).

The modifications in head furnishings observed in the series here discussed coincide very closely with those observed fol-

lowing sinistral ovariectomy. It was pointed out earlier (Domm, '27) that in such cases there is a considerable range of variability in the time at which head furnishings manifest signs of growth. In some instances this became relatively pronounced by the end of the second month, while in others activation may begin much later, depending apparently upon the amount of rudimentary gonad tissue originally present as well as its degree of activation and growth. It was further noted that in some cases there was a slow gradual growth, while others revealed very rapid growth, thus attaining maximum size relatively much earlier. The cases under discussion in this paper revealed practically the same phenomena. Bird no. 889 revealed a very slow, but nevertheless, consistent growth during the first eighteen months following the operation, while during the subsequent sixteen and one-half months to the termination of the experiment there was a consistent increase in size, though somewhat accelerated (see table). Bird no. 885 revealed a gradual increase, attaining maximum size nineteen months following the operation; ensuing, there was a gradual decrease to the twenty-fifth month, then a subsequent increase to the thirtieth month, which, however, did not recover the previous maximum attained at nineteen months. During the final six months, prior to termination of the experiment, there was again a slight decrease, occasioned unquestionably by the fact that the bird became sick during this period. Postmortem revealed no abnormality except a dark, brownish-colored liver (see table). Bird no. 873 revealed a condition in general similar to that of bird no. 885, its head furnishings likewise attaining maximum growth at nineteen months, followed by a recession, but in this case a subsequent increase terminated by the close of the experiment (see table). This case (no. 873), however, differs from the previous in that it revealed minor fluctuations superimposed on the major, referred to above, which are recorded in the protocol, but not revealed in the table.

The remaining three cases (birds nos. 881, 883, and 892) are all characterized by pronounced early growth which was

particularly conspicuous in bird no. 892 (pl. 1). In this case pronounced growth began early in the fourth month following operation, and by the seventh month head furnishings had attained practically their maximum growth. Subsequent major fluctuations are revealed by the table which were interspersed by minor ones which are not revealed. There was a gradual increase from the twenty-fifth month on up to the thirtieth. At the end of this period the comb measured 11.8 cm. in length and 7.0 cm. in height, which was the maximum size it attained during the experiment. During the last four and one-half months there was a slight decrease. The head furnishings of bird no. 883 also revealed early pronounced growth, though they revealed less actual growth than in bird no. 892. Here accelerated growth likewise commenced early in the fourth month. It attained its maximum by the ninth month and thereafter revealed a uniform decline, until it was killed, due to its poor condition, thirteen and one-half months following the initial operation. Bird no. 881, also characterized by early pronounced growth, likewise showed pronounced growth beginning with the fourth month which continued up to the sixth month. Following this there was a general decrease, followed again by a general increase, which terminated thirteen months later at nineteen months. However, the size attained at nineteen months was somewhat less than that at six months. After the nineteenth month there was again a decrease during the ensuing four months, followed by a gradual increase up to the termination of the experiment, at which time head furnishings had attained their maximum size for the experiment (see table). At this time they had probably about attained their maximum size for this growth cycle and had the bird not been killed just then, they probably would have receded again, for the comb showed no change during the last two weeks, while the wattles receded 1 mm. each in length and width.

Discussion

The fluctuations in size of head furnishings noted in the above cases, as well as previously (Domm, '27; see tables), are of interest and require further explanation. No doubt, some of the fluctuations are occasioned by periodic activity of the gonads in these birds. It is conceivable, as revealed by study of other forms, that we are concerned here also with variations in endocrine activity of the gonads which would occasion fluctuations in size. However, there is another fact which cannot be overlooked and that is the pronounced seasonal size variations exhibited. Head furnishings in the majority of cases invariably attain their maximum size during the late winter months and their minimum during the summer, the latter usually aggravated by the molting period. These are major fluctuations which are revealed by all birds in good physiological condition and they are usually punctuated by minor fluctuations. In a bird whose head furnishings show the minimal size in late August or early September there is a gradual increase under normal conditions until the maximum is attained some time in February or March. Following this there will be a general gradual decrease until the minimum is attained late in the summer. At the termination of these increase and recession cycles there are usually short periods when no apparent growth is registered, so that for a time it may be difficult to determine the general trend. These major cycles, if we may call them such, are usually punctuated by minor fluctuations, but the general trend is either increase or decrease. To illustrate more specifically, the general curve from August to February is up, or an increase in size, but this is not always an uninterrupted trend, for in most instances there are minor recessions at varying intervals followed by renewed activity or increase. The same is true from February to August, when there is usually a gradual recession, for there are periods when recession is interrupted by a slight increase which after a time halts and lapses again into a recession.

Gonad activity, no doubt, is an important factor governing these growth cycles, but we are of the opinion that there are other vital contributory factors. One of these probably is available sunlight, for it appears that during periods of decreasing sunlight head furnishings reveal in general a gradual increase in size, while during periods of increasing sunlight there is usually a gradual decrease in the size of these characters. Assuming that the bird receives its actinic rays only, or primarily, through these appendages, increased growth would tend to compensate for decreasing sunlight, and vice versa. An observation repeatedly made which would seem to lend support to the above view concerns confinement. I have repeatedly observed that where birds are confined in individual cages with sunlight inaccessible, they will in time invariably show pronounced increase in size of head furnishings followed by recession when returned to their pens where sunlight is accessible. General activity may be a minor factor tending somewhat to inhibit excessive growth; that is, animals exercising vigorously in the absence of sunlight would tend to develop less comb tissue than those deprived of this activity. This is an interesting problem to which attention was previously directed (Domm, '30 b) and which is being further investigated.

EFFECT ON PLUMAGE

It was earlier pointed out (Domm, '24, '27, '28 a, '29 a and b, et al.) that complete sinistral ovariectomy causes the development of male plumage in the Leghorn fowl. These changes may be observed very early, and in the actively growing feather of the breast, where changes are readily observed due to color contrast, one may find new unmistakable black male basal parts appearing within a few days after the operation. Newly formed feathers in the deplumed areas may reveal these changes in the course of eight to ten days, when their tips will be seen projecting from their respective follicles. These changes proceed until the bird becomes fully male-plumaged, the time varying in different cases, owing to age

and season of operation. The young growing bird undergoes the change more rapidly than a mature bird, while birds operated prior to the molt change more rapidly than those operated shortly after. These changes may proceed in the male direction until the bird becomes fully male-plumaged. However, it was found that birds did not retain this assumed male plumage indefinitely, but that at varying periods subsequently it would again be replaced by the female type (Domm, '24 and '27). We have accumulated evidence showing that this transformation may, in exceptional cases, not set in during the first three years following operation (unpublished data), though in the majority of cases it comes about much sooner. Furthermore, the process of transformation may be a long-drawn-out affair, and it remains a question whether all sinistrally ovariectomized Brown Leghorns revert completely to female plumage. Many of these birds retain a mixture of male, female, and intermediate feathers in varying proportions, occasioned apparently by the fact that the quantity of female hormone produced fluctuates above and below the threshold of effectiveness, alternately permitting and suppressing growth of male feathers. Even in those birds most completely transformed, the tail feathers are the last to revert; hence many of these birds show a more or less complete female plumage except for the tail feathers.

The birds of the present series, in which only left testis-like gonads were present, were in no important respect different in plumage characterization from the large number of birds studied having right testis-like gonads only. Two of the birds developed and retained male plumage throughout the period of the experiment; these were birds nos. 873 and 889, both under observation for thirty-four and one-half months (see table). Bird no. 889 was completely male-plumaged² by five months following the operation. It developed a beautiful

² This term as used here implies some juvenile as well as adult male plumage. Each of the birds developed juvenile male plumage following the operation, but in each case this was practically completely replaced by adult male by six months after the operation. The juvenile breast feathers are always first to be replaced by adult male—a condition which prevails also in the normal male.

coat of male plumage and no other type of plumage appeared during the term of the experiment. Bird no. 873 was completely male-plumaged by six months following the operation except for some scattered old female wing coverts which were retained until the last of the eighth month. This bird also developed a beautiful coat of male plumage with no indication of reversion throughout the period of the experiment.

The history of birds nos. 881 and 885 is very similar in that both revealed a relatively late reversion in plumage. Bird no. 881 was completely male-plumaged by seven months after the operation. It retained a beautiful male coat of plumage until the twenty-first month following the operation, when new intermediate³ back feathers began to appear. New intermediate feathers were found in different areas from time to time during the following five months. However, by the twenty-sixth month new female back and saddle feathers were coming in, though new intermediate of different ages were found on anterior and posterior breast areas, while by the thirtieth month the new feathers were female in all areas. The bird continued to develop new female feathers during the following four and one-half months, when the experiment was terminated. At this time its plumage was predominately of the intermediate type with many scattered female and fewer male (see table). Bird no. 885 was completely male-plumaged by eight months following the operation. It retained a beautiful complete male plumage until the nineteenth month, when new intermediate back feathers began to appear, while by the twenty-second month new female feathers were found on the back and saddle while new on the breast were still intermediate. One month later (twenty-third) new feathers were female in all areas. Only female feathers were developed from this time until the bird was killed thirty-one and one-quarter months after the operation. When the bird was killed, its plumage was female in all areas excepting the tail, which was of the intermediate type.

³ For description of intermediate plumage the reader is referred to an earlier publication (Domm, '27, pp. 70-84).

Birds nos. 883 and 892 revealed a very early transformation from male to female plumage. Neither of them became completely male-plumaged before this secondary transformation set in. In bird no. 883 new intermediate back and saddle feathers made their initial appearance between four and five months following the operation, while by seven months the new were female in all areas and continued such until the thirteenth month, when new intermediate again appeared on the breast, though new back and saddle were still female, while at the time of killing the new back feathers were intermediate and the new breast male. This reversion to male plumage was no doubt occasioned by the poor condition of the bird causing gonad suppression, which lead to its premature killing at thirteen and one-half months subsequent to the operation.⁴ In bird no. 892 (pl. 1) the new plumage was female at five months and continued such until the thirteenth month. At this time intermediate feathers were found in all areas, the new breast feathers were male while those on the saddle were still intermediate, while one month later new feathers were male in all areas. It continued to show new male feathers in all areas during the fourteenth and fifteenth months, but by the sixteenth month a reversion toward female had again set in, for at this time the new back feathers were female and the new saddle intermediate, while new on the breast and wings were still male. By the seventeenth month the new back and saddle were female, while the breast were intermediate, and by the eighth month the new were female in all areas. New female feathers continued to develop up to the twenty-fourth month, when it again began to revert and during the ensuing four to five months the plumage went from female through intermediate to male and back through intermediate to female again, while during the last four months of the experiment only new female appeared.

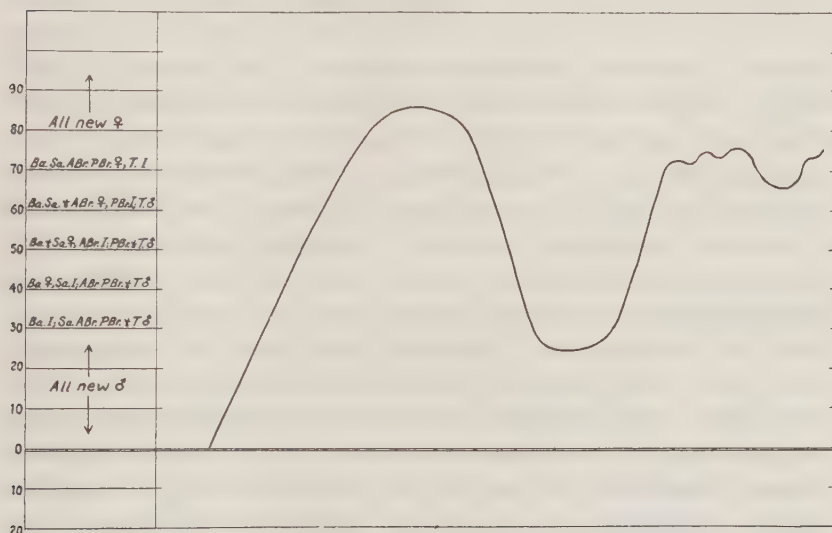
⁴Sickness or poor physiological condition has invariably been found to ultimately bring about this secondary reversion from female back through intermediate to male and is unquestionably due to the fact that there has been a suppression of endocrine activity, which is further substantiated by the fact that disease, if prolonged, may cause noticeable reduction in size of gonads.

Discussion

A point that perhaps may be emphasized and is of particular interest is the fact that these plumage changes are paralleled by changes in size of head furnishings, which, of course, are controlled by gonad activity. Reference to the table will reveal the fact that in each of the cases described the initial reversion in plumage from male to female was preceded by considerable increase in size of head furnishings, which in turn disclosed gonad activity. The secondary reversion from female back to male invariably accompanies or is preceded by a regression in the size of these characters, indicating presumably reduced gonad activity, while a subsequent reversion from male to female will be preceded or accompanied by increase in size of head furnishings, indicating again increased activity. These correlated changes have been frequently observed (compare Domm, '27 and '29 a, b).

An interesting feature that perhaps needs further emphasis concerns the differential in susceptibility to gonadal hormone of the feathers in different body areas which is clearly revealed by some of the above cases as well as in our other ovariectomized birds (Domm, '27). There can be no question about the fact that the feathers of the breast will not revert from male to female as readily, or under the same concentration of female hormone, as will those of the back and saddle areas. Also, in the secondary reversion from female to male the breast area reveals the change before the back and saddle, becoming intermediate while those of the back and saddle areas are still female. This observation has been made repeatedly on large numbers of our ovariectomized fowl. Juhn, Faulkner, and Gustavson ('31) have followed this suggestion and have demonstrated that in the capon there is also a differential threshold for the female hormone between breast, and back and saddle feathers. They have also shown that in the capon there is a difference in growth rate between these two areas, breast feathers growing more rapidly than do back and saddle. That a comparable condition prevails in the poulard has been shown by the fact that the definitive

type of plumage replaces the juvenile earlier on the breast and sides than on the back and saddle (Domm, '27). We believe that the situation can be interpreted simply by the fact that the saddle feathers require less female hormone to effect the change from male to female than do, for instance, the breast feathers. The suggestion of Juhn, Faulkner, and Gustavson that the differential threshold is directly due to the



A diagram illustrating regional plumage changes observed in ovariectomized fowl brought about by the differential threshold of feather areas to female hormone as well as quantitative fluctuations in the production of female hormone. *Ba.*, back; *Sa.*, saddle; *A.Br.*, anterior breast; *P.Br.*, posterior breast (lateral); *T.*, tail; *I.*, intermediate.

differential in growth rate seems plausible and would imply that rapidly growing structures require a greater concentration of hormone to effect a given change than do those with a less rapid growth rate.

The differential thresholds of various feather areas in the poulard can perhaps best be illustrated with the aid of a diagram (p. 231), made on an arbitrary scale, to represent the principle involved. Here the ascending curve represents gradual increase in volume of female hormone produced by

the testis-like gonad of the male-plumaged ovariectomized fowl. As its production increases quantitatively, it finally reaches the line 30 on the ordinate, where it begins to have a suppressing influence on male plumage. The feathers of the back, being most susceptible, or having the lowest threshold, are the first to show the effect by a deposit of female and male pigments in the growing area of an otherwise male feather characterized as intermediate. This process of feminization gradually becomes more pronounced as the quantity of female hormone produced increases; that is, the growing back feathers become more female in character until the quantity of hormone produced is at 40, when the new back feathers are completely female. During this period reversion of plumage has been confined to the back area. However, as the quantity of hormone produced increases it gradually goes beyond 40, which is the threshold for the feathers of the saddle area. The new saddle feathers will thus begin to show some female parts, or intermediacy, while the new back are completely female. As the quantity of hormone produced increases from 40 to 50 the new saddle feathers are undergoing the same gradual change from male to female as did the new back between 30 and 40. When the point 50 is attained, new saddle are also completely female, as the back have been since point 40 was attained. However, at 50 the threshold of the anterior breast region is attained the new feathers of which are now showing intermediacy, while at 60 the new feathers of this area will be completely female. At this point the threshold of the posterior breast area is attained, hence the new feathers of this area show intermediacy and are undergoing the same gradual change⁵ from male to female as did the back feathers between 30 and 40. When the quantity of hormone produced

⁵ These changes frequently are difficult to detect in consecutive areas unless one takes the precaution to force simultaneous regeneration, since one cannot always find new feathers in all areas in the same stages of development. New feathers varying in age in consecutive areas may be intermediate, giving the impression of simultaneous intermediacy when in reality new feathers of comparable ages at the time may be female on the saddle, intermediate on anterior breast, and male on posterior breast.

reaches the point 70, new posterior breast feathers will be completely female and beyond this point all new body feathers excepting those of the tail are female. The tail feathers are the last to revert (Domm, '27) and we can conceive of them as reverting also when the quantity of hormone produced goes beyond 80. We can logically assume, on the basis of extensive observations in ovariectomized fowl, that the quantity of hormone produced rarely goes beyond the line 80, but rather that it fluctuates between 70 and 80, since tail feathers more rarely revert completely. It also is obvious that it does not continue to fluctuate between 70 and 80, but that it may from time to time recede below 70 or more exceptionally even below 30, as shown by the secondary reversion of plumage from female to male in all areas. Allow us to assume that the volume of hormone produced has been above 70 for a sufficient period to produce female feathers on all but tail areas. As it recedes below 70 the new posterior breast feathers, being most resistant, or having the higher threshold, immediately reveal their insubordination by becoming intermediate, and when it has receded to 60 they are entirely insubordinated and again become completely male. At this point the less resistant new anterior breast feathers begin to show insubordination as did the new posterior breast feathers at 70, and below 50 they also will have become completely insubordinated, now being completely female. Thus if hormone production recedes below 50, the saddle feathers are affected and if below 40, the back in the same manner as were the anterior breast between 70 and 60. Below 30, then, the feathers in all areas have become completely insubordinated to the suppressing influence of the female hormone as revealed by new ingrowing male feathers. A subsequent rise in hormone production would again cause a reversion to female plumage in the same manner as did the initial rise which is illustrated by some of the cases described. Thus, as clearly indicated by the diagram, as the quantity of female hormone produced increases after ovariectomy the feather areas with the lowest threshold are affected first and so on in their order up to the highest,

while, assuming that the quantity of hormone produced has reached the point 80 where all areas have reverted, upon recession the order is reversed, areas with the highest threshold⁶ being first affected. Also, the order of change for any particular area during hormone increase is the reverse of that during decrease, the former being male; intermediate, female, and the later female, intermediate, male.

If the loss of female hormone immediately following ovariectomy were as gradual a process as is its increase or decrease in the later life of many poulards, then it would unquestionably be registered in a similar manner in the growing feather. In fact, there can be no question but what the effect of hormone loss following ovariectomy becomes apparent earlier in some areas than in others because of their differential thresholds, but since its complete loss is merely a matter of hours, the effect appears to be simultaneous in all areas. This differential could be easily determined in such cases if one were to reintroduce full concentrations of the vanishing hormone at intervals and follow the effects on growing feathers. One would then no doubt find that, for instance, by reintroducing the hormone at thirty hours after ovariectomy, sufficient hormone had been lost to affect some areas and not others.

The fluctuations in hormone production which occur in many ovariectomized birds prevent the continuous uninterrupted production of any one type of feather. It should be pointed out that in some instances these fluctuations in hormone production are not revealed. This may be due to the fact that either they do not occur in such cases or, what is more probable, that the quantity of hormone produced is considerably beyond the threshold of effectiveness, or beyond 80 on the ordinate of the diagram, so that fluctuations do not recede below 80, where they would become apparent in new plumage being developed. Such cases are the small number

⁶ Our evidence shows that the thresholds of the areas discussed are not all separated by the same number of units as the diagram would indicate. The thresholds of back and saddle areas, for instance, are not as widely separated as are saddle and anterior breast area. Also, the evidence from certain cases seems to show that the threshold of wing coverts, not given in the diagram, may be near that of the anterior breast area.

of ovariectomized birds which have completely reverted to female plumage and have become more or less stable in this condition. Prolonged sickness may bring about a secondary reversion back through intermediate to male in all areas in such cases. These fluctuations are also revealed by changes in the size of head furnishings.

In following these plumage changes feathers are periodically plucked from definite body areas and filed for record (Domm, '27). In this manner new feather growth is stimulated and definite indicators of hormonal conditions are thus available at all times. However, some new growing feathers may be found without periodic plucking at practically all times. Indications, however, are that feather follicles in general do not have an unlimited capacity for regeneration between successive molts, though exceptions occur as well as regional differences, so that it becomes necessary to pluck the feathers from different follicles of the same body area at varying periods.

The gonad-plumage interrelation obviously may be explained quantitatively. The female completely deprived of all gonad develops and retains male plumage. Small masses of gonad of female origin do not alter this condition (Domm, '29 and '29 a), however, as they increase in volume and presumably also in hormone production male plumage is suppressed in the manner described above. This suppression of male plumage is explained by the secondary gradual production of female hormone by these testis-like gonads. In our previous studies (Domm, '27) we called attention to the presence of fluid-filled nodules of varying size on many of these testis-like gonads similar to those frequently found on normal left ovaries. One also frequently encounters firm yellowish yolk-like nodules well embedded or projecting from the surface of the testis-like gonad. These we believed were due to the development of an abortive cortex by the testis-like right gonad. These observations anticipated the recent findings of Gray ('30), who made a careful histological study of considerable of our hypertrophied right-gonad collection. He found

histological evidence in a number of cases of the secondary activation of the germinal epithelium giving rise to cortical elements. It is therefore logical to assume that the suppression of male plumage in the poulard is due to the elaboration of an ovarian hormone by these tissues.

EFFECT ON SPURS

The normal female of our breed does not have spurs, though a few exceptional cases in which an otherwise normal female revealed well-developed spurs have been recorded (compare Goodale, '16; also Domm, '27, p. 86).⁷ The young Leghorn pullet invariably shows spur rudiments as small oval scales embedded on the inner surface of the shank. These rudiments gradually become somewhat more conspicuous with age, but in no instance have they ever assumed the characteristics of a developing spur.

The complete sinistrally ovariectomized Leghorn has invariably developed spurs in our experiments. True, these have varied in rate of growth and ultimate size attained, though conspicuous well-developed spurs are constantly found by ten months to one year following operation (Domm, '27). The development of spurs in the birds of this series seemed to differ in no wise from those completely sinistrally ovariectomized with right testis-like gonads. The longest spurs were found in birds nos. 892 and 873 (see table). Those found in birds nos. 881, 885, and 889 were somewhat less developed than the former and reference to the table will reveal that they were all approximately the same size. The spurs of bird no. 883 measured 1.8 cm. at death thirteen and one-half months following operation, at which time the spurs of bird no. 873 measured 2.3 cm.; no. 881, 1.4 cm.; no. 885, 0.9 cm.;

⁷ This condition has been exceptionally rare in our flock of Brown Leghorns, only two normal, spurred, females having thus far been encountered. The second case recently found had two well-developed, symmetrical spurs measuring 1.2 cm. in length when eleven months old. The condition, no doubt, is more common in some flocks than in others. Dr. A. W. Kozelka states that spurred females were not infrequent in the White Leghorn stock from which some of his birds were derived.

no. 889, 2.0 cm., and no. 892, 2.1 cm. (from protocol not given in table). The spurs of bird no. 885, killed thirty-one and one-quarter months after operation (see table), also would probably have been somewhat longer had the bird been killed three and one-quarter months later, as were birds nos. 873, 881, 889, and 892. For data on spur growth following sinistral and bilateral ovariectomy the reader is referred to previous publications (Domm, '27 and '29).

EFFECT ON VOICE AND BEHAVIOR

The differences in behavior exhibited by the sexes in domestic fowl probably are well known and need very little description. Such common masculine characters as crowing, treading, combativeness, leadership, summoning female to feed, issuing warning call, circling wing, and pursuing female, or such feminine characteristics as laying, cackle, broodiness (in some birds), non-combativeness, acquiescence need scarcely be mentioned.

Following complete castration in the male, there is a loss of all dependent sex characters. Exceptions have been repeatedly noted, though these can probably in most instances be explained on the basis of incomplete operation or they may be postpuberal castrates in which masculine behavior has not yet disappeared. Quite apart from the above probable causes of exceptional masculine behavior in the capon, it appears that segregation with females in the absence of the male may in exceptional cases elicit such masculine responses as crowing and treading.

The complete sinistrally ovariectomized female (Domm, '27) may ultimately develop a more or less complete masculine behavior. Such birds may exhibit all the genuine masculine characteristics enumerated above, though only one has ever been actually known to tread. Complete bilateral ovariectomy brought about the loss of acquired masculine behavior (Domm, '27 and '29 a). The behavior of the birds of this series with only left testis-like gonads differed in no wise from birds sinistrally ovariectomized and exhibiting only right

testis-like gonads. Bird no. 892 was known to crow, fight, attempt treading, summon the female, and issue the warning call. Birds nos. 873, 881, 885, and 889 were all masculine in their reactions, though they were not known to crow, but may have done so. Bird no. 883 was killed before any attempt had been made to definitely observe its behavior. Hence the effects of right and left testis-like gonads on behavior reveal that they are potentially equivalent.

EFFECT ON SIZE

The difference in size between the sexes of the Leghorn is not exceptional in domestic fowl, where the male quite generally exceeds the female in size and weight. Early castration in the male results in the development of a bird larger and heavier than the normal. Complete sinistral and bilateral ovariectomy does not seem to produce comparable modifications in the female (compare Domm, '27 and '29 a). Skeletal preparations are being made to determine actual size differences in various sexual types. Size differences as determined by actual body weight reveal no marked deviations between ovariectomized birds developing only right or left testis-like gonads.

DISCUSSION

It is therefore evident that the characterization of the poultard is similar whether she reveals a right or a left testis-like gonad. In either instance the head furnishings may become masculine in size and texture. The plumage which becomes male soon following ovariectomy may ultimately revert to the female type in either case. Both types develop comparable spur tissue. No differences could be observed in their behavior, those with only left testis-like gonads became as masculine as those with only right. It was found that the wolffian ducts may be stimulated to the same degree by either gonad. No differences were found in the oviducts of either type.

The cases discussed were not the product of an intentional decortication of the ovary, but were selected from a group of

bilaterally ovariectomized poulards which had been incompletely operated on the left side and were subsequently found to be developing left testis-like gonads. The condition could no doubt be experimentally produced in birds in a large percentage of cases by decortication without subsequent cauterization. The application of the cautery following sinistral ovariectomy in the fowl unquestionably destroys much potential testicular tissue.

Our previous studies (Domm, '24, '27, '28 a, et al.) have shown that the female fowl possesses tissues in the rudimentary right gonad which are comparable in their effects, on the dependent sex characters, to the endocrine cells of the testis. The present experiments prove conclusively that the normal left ovary possesses similar tissues also which are comparable in their effects. In both gonads of the hen these tissues are normally suppressed by the cortex of the left ovary. Following the removal of the left ovary these tissues, which normally comprise the entire right gonad, hypertrophy, or, if only the cortex of the left ovary is removed, these tissues are allowed to hypertrophy in both gonads and commonly do so (Domm, '27). Poulards having testis-like gonads on both sides simultaneously differ in no important respect from those having such organs either on the right or on the left side only. The results of feminization experiments in the fowl indicate that under such conditions the medullary tissues of the left ovary may become activated in the presence of the cortical tissue and give rise to a tubular testis-like tissue (Greenwood, '25; Benoit, '26; Caridroit, '26). The similarity in structure between such testis-like tissue developed in a grafted left ovary and that usually found in the sterile hypertrophied rudimentary right gonad was emphasized by Zawadowsky ('26). The endocrine effects of such grafted ovaries may be comparable to those of the hypertrophied rudimentary right gonad. The masculinizing effect of the activated medullary tissues is revealed in the growth of head furnishing and behavior, while the feminizing effect of cortical elements is revealed in the development of female plumage (Domm, '28 b).

The formation of testicular tissue on the site of the removed left ovary may be briefly explained on the basis of the known embryological development of the normal ovary. Embryologically, the medulla of the ovary is the homologue of the testis. Normally, development of the right rudimentary gonad of the hen does not continue after the medulla is formed. It ordinarily remains in this embryonic condition throughout the life of the fowl. However, following ovariectomy the medullary tissue of the right gonad becomes activated and gives rise to a testis-like gonad. If the operation is performed soon after hatching, before the primordial germ cells have disappeared, a testis with fully differentiated motile spermatozoa may result (Domm, '29 b, '30 a). Embryonic growth of the left gonad does not cease at the same stage of development, but continues and forms the cortex also, which is the functional part of the ovary. The cortex normally inhibits the growth of the medullary tissues of either gonad, as pointed out above, while its removal permits their growth into testis-like tissue. Whether the medulla of the left gonad can give rise to functional testicular tissue under certain conditions, as the right has been found to do, remains a question, though we see no reason why it should not if normal primordial germ cells were present following decortication.

SUMMARY

1. The purpose of the present experiment was to demonstrate the equivalent potentialities of the left testis-like gonad, not infrequently found in sinistrally ovariectomized fowl, and the hypertrophied testis-like rudimentary right gonad almost invariably found in such birds.

2. The birds described were bilaterally ovariectomized individuals which subsequently developed prominent left testis-like gonads on the site of the removed left ovary with no trace of the destroyed right gonad.

3. The wolffian ducts, slender threads difficult to find in the normal female, in each case revealed readily perceptible growth and in some became definitely convoluted.

4. The oviducts were all considerably below that of the normal in size. They varied a great deal, though the larger ones were found in birds having undergone a reversion to female plumage and the smallest in those birds showing no indications of such a reversion.

5. In the presence of the left testis-like gonads the head furnishings may become large and masculine in character. Variations in size were found from time to time, interpreted as due to their dependence upon the size and condition of the left testis-like gonads.

6. Following the operation the plumage became male, but subsequently, varying greatly in different individuals, it reverted to the female type. In some of these individuals there were secondary reversions to male followed by reversions back to female. Such changes were interpreted as due to fluctuations in the production of female hormone, the volume of which consequently ranged above and below the threshold of effectiveness for different feather areas. In two cases the reversion from male to female had not yet set in at the termination of the experiment.

7. All individuals in the series revealed some masculine behavior, which was particularly pronounced in one case. None of the birds were actually known to tread.

8. Well-differentiated spurs were exhibited by all individuals, though the ultimate length attained varied somewhat in different cases.

9. All modifications observed in the birds of this series revealing only left testis-like gonads coincide very closely with those previously observed in sinistrally ovariectomized birds revealing only right testis-like gonads, and demonstrate quite conclusively the equivalent endocrine potentialities of right and left testis-like gonads in ovariectomized fowl.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

No. 892. Poulard bilaterally ovariectomized. For detailed history see table. This bird was hatched on June 30, 1926. A sinistral ovariectomy was performed on September 17, 1926, when the bird was seventy-nine days old. A dextral ovariectomy was performed eighteen days later on September 5th, destroying the right rudimentary gonad by electric cauterization. The photograph was taken on May 21, 1929, two years and eight months following the initial operation, and reveals typical poulard characterization. The plumage at this time was predominately of the male type, with many intermediate and some female feathers. The new ingrowing feathers were of the female type. Head furnishings were large and masculine in character. Spurs were well developed. Behavior was masculine. A dextral laparotomy performed on November 5, 1927, revealed no right gonad tissue, though left testis-like gonad could be seen. The bird was killed on July 30, 1929. Postmortem examination revealed no right gonad, while a prominent left testis-like gonad was found (fig. 892, pl. 2).

No. 1050. Poulard sinistrally ovariectomized. This bird was hatched on May 23, 1927, and sinistrally ovariectomized on May 28, 1927, when five days old. The photograph was taken on June 11, 1930, three years and fourteen days following the operation. The plumage at this time was predominately of the intermediate type, with many scattered female and fewer male feathers indicating a gradual change from the male to the female type. It revealed prominent masculine head furnishings and well-developed spurs. The bird was not known to crow, though otherwise it revealed a characteristic poulard masculinity. It was killed on June 20, 1930. Postmortem revealed no left gonad; however, a characteristic testis-like right gonad was found.

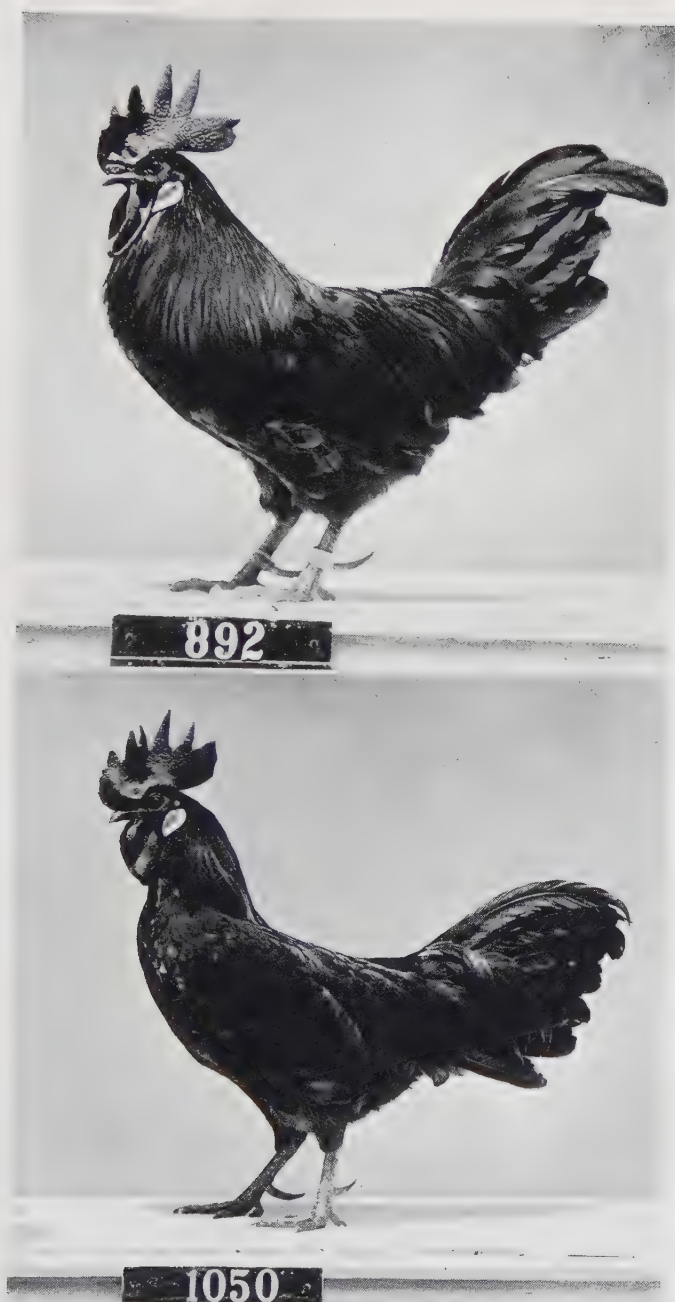


PLATE 2

EXPLANATION OF FIGURES

Case no. 1164, showing right compensatory gonad, right and left convoluted vasa deferentia, and left oviduct. No trace of left gonad could be found indicating complete ovariectomy. This poulard was hatched on June 16, 1927, and sinistrally ovariectomized July 10, 1927, when twenty-four days old. It was killed on May 13, 1928, 308 days following the operation. *Adr.(L)*, left adrenal; *ao.*, aorta; *cl.*, cloaca; *f.*, fat; *ti.(Rl.T.)*, testis-like right gonad; *K.*, kidney; *m.d.s.*, median dorsal mesentery; *Or'd.*, left oviduct (rudiment of right oviduct obscured); *ur.*, ureter; *Rt.v.d.*, right and left vas deferens; *vf.*, femoral vein; *r.r.*, renal vein; *r.r.p.*, renal portal vein.

Case no. 892 (see table), showing prominent left testis-like gonad with convoluted right and left vasa deferentia and left oviduct. No trace of right gonad could be found, this having been completely destroyed by cauterization.

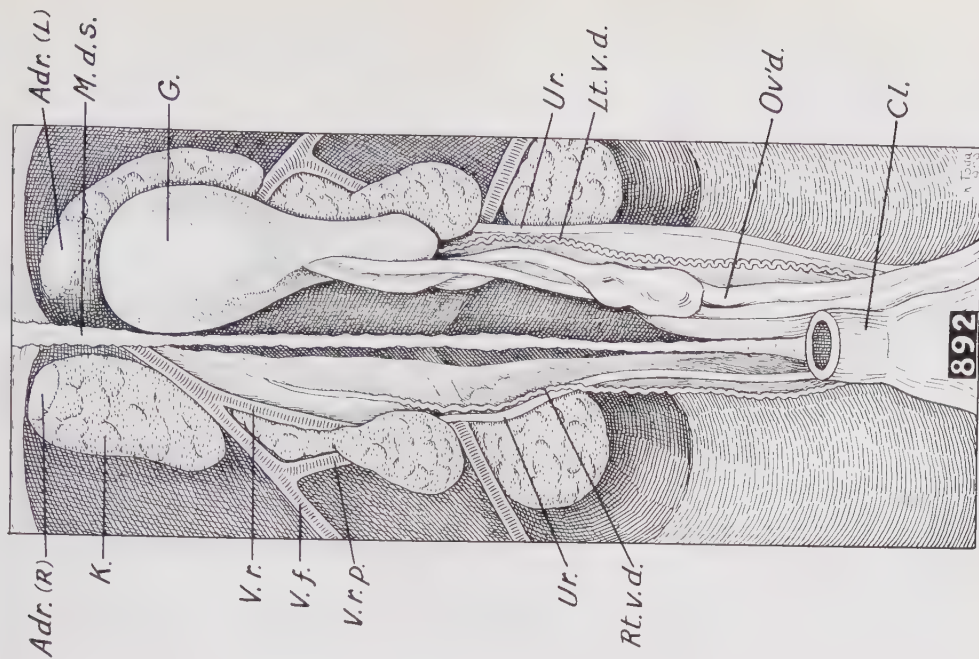
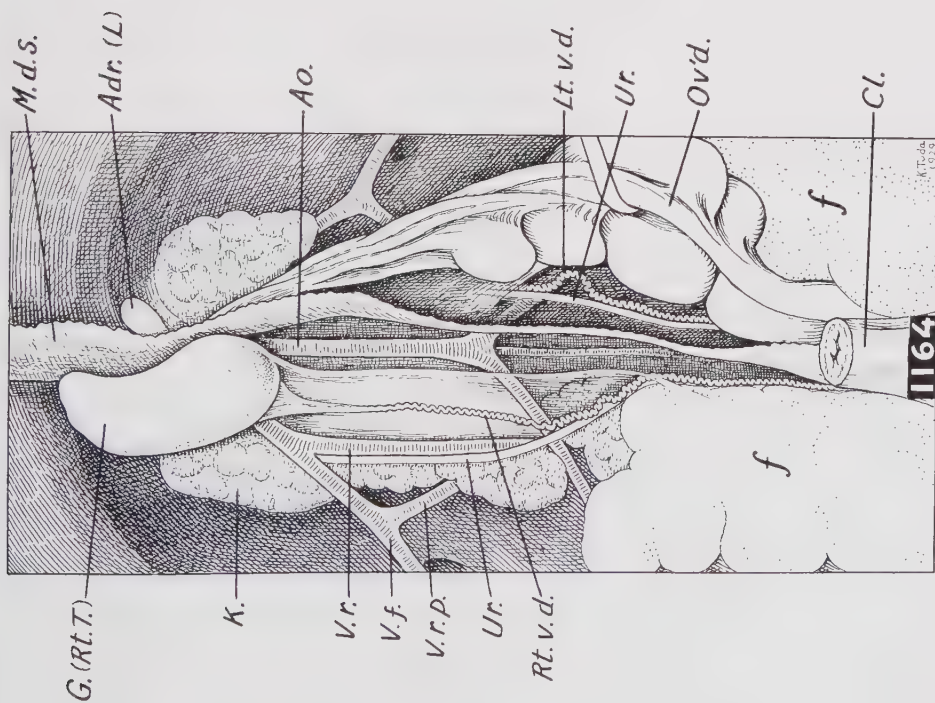
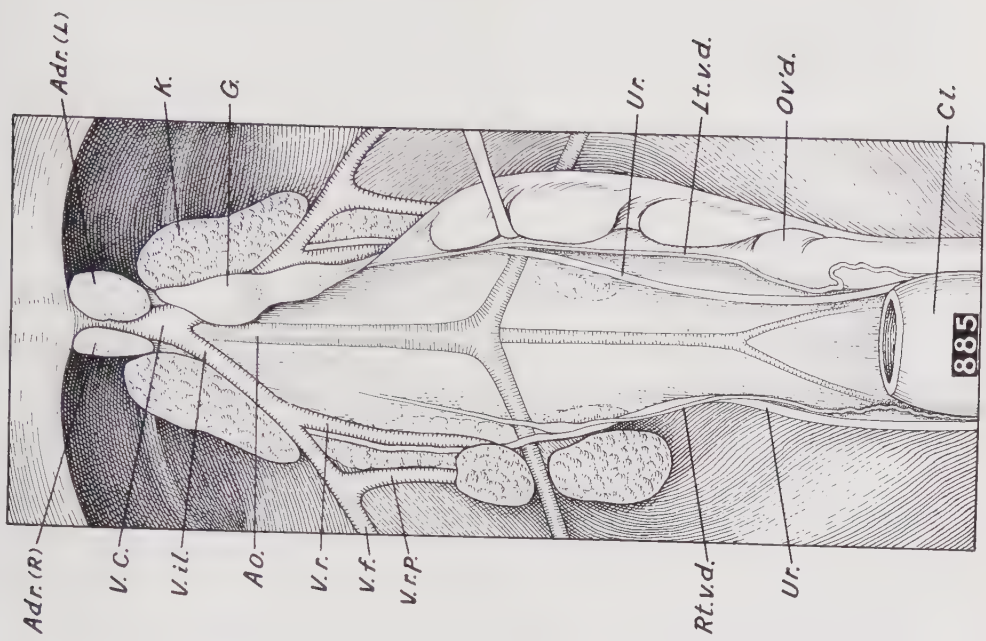
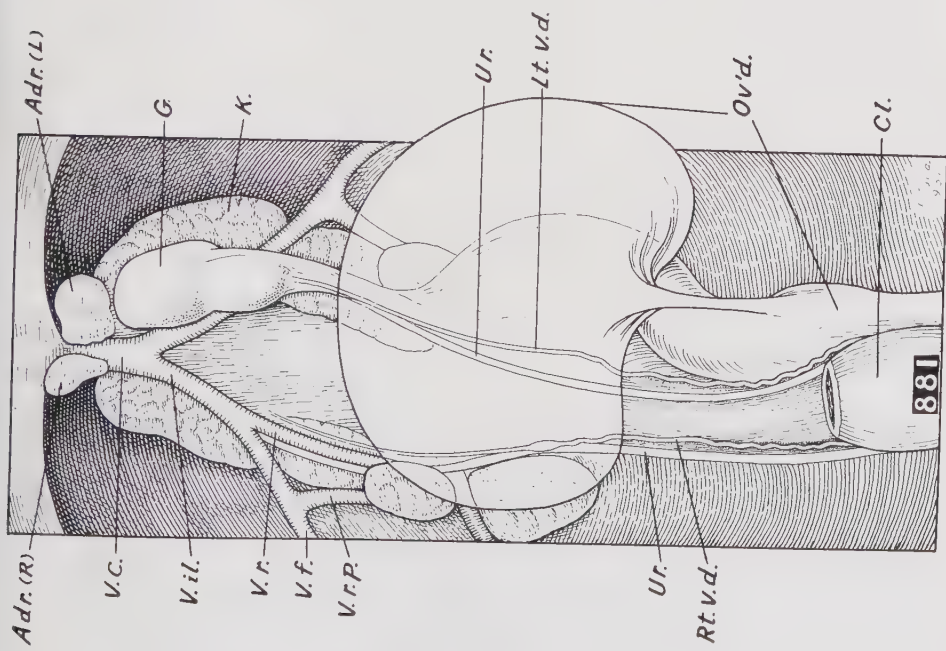


PLATE 3

EXPLANATION OF FIGURES

Case no. 881 (see table), showing left testis-like gonad with slightly convoluted right and left vasa deferentia and left oviduct. The distended portion of the oviduct, shown in outline, represents a fluid-filled cyst. *Adr.*, right and left adrenal; *cl.*, cloaca; *G.*, testis-like left gonad; *K.*, kidney; *Ov'd.*, left oviduct (rudiment of right oviduct obscured); *ur.*, ureter; *Rt.v.d.*, right and left vas deferens; *v.c.*, vena cava (postcaval); *v.f.*, femoral vein; *vil.*, iliac vein; *vr.*, renal vein; *vr.p.*, renal portal vein.

Case no. 885 (see table), showing left testis-like gonad with right and left vasa deferentia, both slightly convoluted posteriorly, and left oviduct. No trace of right gonad was found in either of these cases.



A REVIEW OF THE HISTORY OF THE CENTRIOLE IN THE SECOND CLEAVAGE OF ASCARIS MEGALOCEPHALA BIVALENS

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TWO PLATES (THIRTEEN FIGURES)

INTRODUCTION

Examination of the literature concerned with the central bodies as found in the cleavage stages of *Ascaris megalocephala bivalens* shows a great diversity of opinion concerning their morphological relationships and their function. The situation assumes added significance when considered in the light of recent work on other forms, such as *Echinarachnius* or *Cerebratulus*. Especially is this true when the problem becomes limited to challenging the actual presence of a centriole as a definite body.

In a study of the literature confined to the centrioles as found in cleavage stages of *Ascaris* both sides of the problem are discussed and definite conclusions advanced. In an attempt to come to some conclusion on the subject as a possible contribution to a general understanding of the mitotic mechanism, the author has studied the process as it occurs in the second cleavage of *Ascaris megalocephala bivalens*.¹ The second cleavage was chosen because in a previous study of chromatin diminution (Fogg, '30) many slides had been prepared from different worms under constant environmental conditions. Additional material has been available and uti-

¹ The original results of this investigation were reported before Section F of the American Association for the Advancement of Science at Cleveland in December, 1930. Continued investigation confirms the original report, which will be included in this article.

lized. Furthermore, it is well known that in this cleavage the two cells, AB and CD, do not divide synchronously. This allows, fortunately, a better opportunity to study the mitotic mechanism where time comparisons may be made. The investigation is not complete, but may emphasize some facts observed by earlier workers in the field and provide suggestions for further research.

METHODS

The methods followed were the same in general as those described in a previous paper (Fogg, '30). In the preparation of some of my material eggs were allowed to develop in distilled water with no apparent effect on the normal activities of the egg. Other solutions were used, such as concentrated HCl and NaOH, but no pictures were drawn from preparations so treated, and so discussion of this phase of the work will be reserved until a later time. As is well known, living *Ascaris* eggs placed in strong Flemming's or Bouin's fixing fluid will continue to cleave and produce fully formed worms inside the shell. In order to allow the fixative to penetrate, an attempt was made to prick the shell. Although this work has not yet been entirely successful, the possibility should give interesting results and further work is intended along this line. A modification of the use of Bouin's fixative was tried by shaking some eggs in chloroform for no longer than one minute previous to placing them in the Bouin fixative. This process allowed a slow penetration of the fixative and some eggs were satisfactorily fixed. It was discovered that by putting the eggs in Flemming's strong or formol-alcohol-acetic fixatives some of the eggs did not continue to cleave, but were killed and fixed. As in the case of the chloroform-Bouin fixative, an examination of the material showed eggs in various stages of development.

The history of the centrioles as demonstrated by the use of these fixatives does not alter the general picture as seen when the Gilson-Carnoy fixative is used. The greater part of the material was fixed in Gilson-Carnoy. Sections were cut at 8μ

and, after the usual procedure, stained with Heidenhain's iron haematoxylin, safranin, Auerbach's acid fuchsin-methyl green, or the Feulgen reaction. Heidenhain's iron haematoxylin gave the best results consistently. In safranin the centrioles stain but faintly. With the Feulgen reaction or with Auerbach's acid fuchsin the stain is not specific.

Through the kindness of Dr. F. F. Lucas, of the Bell Telephone Laboratories, it was possible to obtain ultraviolet photographs of living *Ascaris* eggs, of eggs fixed but unstained, and fixed and stained preparations. The method by which this is done is described by him in an article on cell architecture (Lucas, '30).

OBSERVATIONS

The cleavage stages in different species of *Ascaris* have been most carefully described by many workers (Van Beneden, Boveri, Zur Strassen, Romeis, Meyer, Erlanger, Kostanecki and Siedlecki, Dostoiwsky, Walton, and others). My description, based on observations of original slides, in no way detracts from their extensive observations, but contributes among other things a confirmatory note to the presence of a stained cytoplasmic object which appears as a period-like granule (double in some stages). A brief review of my observations on *Ascaris megalocephala bivalens* follows.

The last demonstrable appearance of a centriole in the first cleavage shows a minute basichromatin-staining granule which does not take the stain intensely. In figure 1 such a stage has been drawn which shows centrioles of different sizes seemingly embedded in a more dense irregularly radiate cytoplasm.² A feature notably common in these stages is the consistent picture of the cytoplasm after the fixative used.

² The words centriole, central body, and centrosome are used in different ways in the literature, as has been pointed out by Wilson in "The cell." In order to avoid further confusion, it will be understood that a centriole is a minute cytoplasmic component which often is in turn surrounded by a homogeneous area called the centrosome, similarly bounded by a radiate configuration called the astral rays.

At the two poles the cytoplasm is always more dense, while in the middle region there is evidence of considerable vacuolization. The reason for this is unknown, suggesting only a possible regional difference in consistency of the cytoplasm at this particular stage. The position of the centriole (double?) is invariable at this stage and has a direct bearing on the question of its continuity. Observations here include stages that suggest doubling of the centriole. Due, however, to the difficulty with which the area stains, only the most readily demonstrable stages are illustrated.

Immediately following the interkinetic period the reappearance of granules can be demonstrated invariably. There are now two granules close together, each embedded in a centrosome (fig. 2). In the cases in which it was impossible to demonstrate the centriole through the interkinetic period the two centrioles always reappear in the region or position which exactly corresponds to the position of the centriole at the late telophase of the preceding division. This is not complete evidence of continuity, but strongly suggests it. Recently, observations on the cleavage stages of the eggs of *Drosophila* (Huettner, unpublished) have revealed without question a demonstration of complete continuity of the centrioles. The same is true in the spermatogenesis of *Ascaris megalocephala*, as has been indicated by Sturdivant (unpublished).

It is well known that varying the staining technique will give different results. For example, if the slides were stained by the short method with Heidenhain's iron haematoxylin, the centrioles were not consistently demonstrated. Staining with the long method and only destaining slightly, the centrioles could be consistently demonstrated. As is seen in figure 2, the centrioles appear as only minute granules and the centrosome is correspondingly small. One concludes from this, then, that the centrioles do not take the stain intensely at this stage. It is a bit of cytologic evidence, however, that early in the mitotic history there is a substance in the cell which reacts differently than does the surrounding area and will take a haematoxylin stain. There are no other granules

in the cytoplasm of the same size or shape or staining reaction which could in any way be interpreted as a substitute for a centriole. That the centriole is not a random granule is also apparent from evidence in subsequent events.

A study of the later stages previous to the metaphase (on observations of over a thousand cells) reveals a series of events remarkably constant and striking and consistent with the observations of all the earlier workers. The centrioles increase in size, progressively acquire the capacity to take the stain more intensely, and there is an apparent migration toward the poles. Figures 2, 3, 4, and 5 show the migration from the time of the first appearance until they reach the poles. A size comparison can also be made from the same figures. It is to be noticed that the fixed appearance does not show uniformity in shape, although the centrioles were carefully drawn with the aid of a camera lucida. There is a coincident increase in size of the centrosome and astral configuration which suggests a possible relation morphologically, but there is no specific evidence of this.

The metaphase in the second cleavage of *Ascaris megalocephala* demonstrates with striking clearness what is commonly described as a centriole. Figure 5 is an example of a typical metaphase in the second cleavage, showing the position and size of the centrioles. The variation in size (compare figs. 5, 8, 10) is dependent upon the amount of destaining. Using the iron-haematoxylin stain with only a slight degree of destaining, very large centrioles may be demonstrated, even larger than those in figure 5. Destaining to a greater extent, they may be made to appear smaller (fig. 7) to very small (fig. 10). Beyond this point, however, they maintain their identity, until the stain is completely lost. In no case, with this fixative or with the limited use of other fixatives and stains, have I ever been able to show the dual nature of the centrioles of this stage as demonstrated by Boveri ('02). The centrioles in most instances give the appearance of being an area or region that is different from the surrounding cytoplasm or centrosome either physically or chemically or both.

Other tests may be applied, however, which tend to confirm the above. It can definitely be said that the astral rays lose their identity before reaching the centriole. The centriole takes a different stain than that of the surrounding medium. It is to be emphasized that there is no clearly defined shape to the centrioles. It may be that they are not bounded by a delimiting membrane. In fact, they may represent a region that is specific chemically when acted upon by a fixing agent or at least sufficiently different from the centrosome to take the haematoxylin stain. In the case of *Ascaris* the centriole appears as a definite unit, although it is conceivable that it might not be so concentrated in other forms and might give a more diffuse appearance when fixed and stained. The variation of the fixative does not confuse the interpretation in *Ascaris*, for in all of the fixatives used the centriole was apparent always, with little variation in size or shape.

The anaphase centrioles progressively lose their affinity for the stain—a condition just the reverse of that found in the prophase. In some instances the centrioles seem to elongate (figs. 7, 8, 9, and 12). In others they are irregularly ovoid (fig. 7). It should also be noted that whenever an elongate centriole is found, usually the centrosome is likewise somewhat more elongate in the same plane. Whether this is of any significance indicating a morphological relationship is not as yet determined. The duality of the centrioles at this stage, so thoroughly demonstrated by Boveri ('02), was not apparent in my material with any of the fixatives used. Many slides containing elongate centrioles in the anaphase were studied and in some few instances, after careful study, indications of a possible transverse split were visible. Destaining the same material gives quite the opposite impression, since the object appears as a unit with no evidences of duality (compare figs. 11 and 12). The same is true with all fixatives used.

A study of the late anaphase and the telophase does not bring out any additional feature. The centrioles progressively lose their affinity for the stain (fig. 11). There is no evi-

dence of duality (figs. 11, 12, and 13). An interesting point is that, while the centrioles are losing their affinity for the stain, the centrosome and astral rays are as conspicuous as in the metaphase.

The interkinetic period is the critical stage, and at the same time the most difficult, in which to demonstrate the continuity of the centriole. In the second cleavage, as found in *Ascaris megalocephala*, the evidence cannot be said to be positive, but it is definitely suggestive. Boveri ('02) was able to show complete continuity. Recently, Huettner (in press) demonstrated perfect continuity of the centrioles in the cleavage stages of the fruit fly *Drosophila melanogaster*. In his case the centrioles are unmistakably clear in the interkinetic stages with entire absence of astral rays. In my material in most cases the centriole entirely loses its affinity for the stain. In a few favorable instances evidences of division can be seen. In the very early prophase of the subsequent cleavage the position of the now-double centriole reappears in exactly the same locus as that from which it disappeared. The sequence of events is so close and the evidence so apparent that there is little doubt concerning continuity; but the point, as demonstrated in my material, cannot be stressed too rigorously.

The ultraviolet photographs of the living cell were not clear enough to differentiate the centriole or the astral configuration. The photographs of the fixed but unstained cells appeared similar to photographs taken with visible light. The fixed and stained slides did not show the centriole to be the focal point of astral rays.

DISCUSSION

No attempt will be made here to discuss the central-body situation as a whole, for the subject has been carefully considered by many workers. (For a general review, see "The cell," by E. B. Wilson.) A review of the history of the mitotic mechanism as it occurs in *Ascaris megalocephala* shows a centriole to be present. In the light of the recent statements concerning central bodies in general (Fry, '29), it

may be apropos to review briefly some of the literature concerning central bodies in *Ascaris megalocephala* variety *bivalens* and *univalens*.

A partial list of observers would include Van Beneden and Neyt, Kostanecki and Siedlecki, Brauer, Heidenhain, and Boveri. All of the above workers recognized the centriole as a definite component in the cell. In fact, a careful review of the literature shows that without exception centrioles have been reported in the early cleavage stages of all species of *Ascaris*. The less recent principal controversial points in reference to the centrioles were well brought out by Boveri ('02) and will only be stated in passing here. Boveri ('02) described the centrioles as minute granules that were double in the metaphase and which maintained their continuity through the subsequent interkinetic period. Van Beneden and Neyt ('87) disagreed only in the structure and size of the centrosomes (centriole) and as to the exact time of division. Kostanecki and Siedlecki ('97) did not show as minute a centriole as did Boveri and thought the size of the centriole to be dependent within limits upon the extent to which the stain is extracted. In the anaphase they showed heavy disc-shaped centrioles which possibly could separate or be pulled apart in later development. Erlanger ('96) showed clear photographs of centrioles, and it is interesting to note that he diagrams a possible picture of the finer structure of the centrosome and aster. This diagram illustrates fine rays approaching the very center of the configuration—in other words, to the centriole. The conception of the centriole as the focal point of rays was indicated, therefore, over thirty years ago. Fürst advanced the theory that possibly the centrioles were only artifacts. He based this claim on the belief that the centrioles could be entirely destained. He does not state, however, what might cause the formation of the centrioles. Romeis ('13) pictures centrioles after Benda fixation with few or no rays present in the configuration. It is evident, therefore, that puzzling questions concerning the centriole have for many years interested the cytologists. The general agreement at

present is that they are not the focal points of rays and do exist as a definite cytoplasmic component in *Ascaris megalocephala*.

It might be proposed that the differences in the above results were due to different methods of fixation. An examination of the literature again shows that many different fixatives were used by each worker. Boveri, for example, used various combinations of acetic acid with picric acid or with alcohol, sublimate, chromic, and osmic fixatives. Fürst employed nine different fixatives; Kostanecki and Siedlecki, three—sublimate, acetic, and picric combinations. Erlanger used glacial acetic and alcohol, Perenyi's fluid, piero-acetic and nitric acid plus alcohol. My own results were based on Gilson-Carnoy, Bouin, Flemming, and formol-alcohol-acetic fixatives. The above is not a complete list, but is sufficient to demonstrate that the observations were not based on one or a limited number of fixatives.

An analysis of the above results does not bring out vital contradictory points. In all cases there is a centriole. The points at issue are the size and shape of the centriole, whether or not it is the focal point of converging rays, and when division takes place. The recent work of Fry ('29) on the echinoderms has again called into question the presence of a distinct centriole and has suggested that the latter may be merely the focal point of astral rays or possibly a random granule.

My observations add but little to that of previous workers. In the form with which I worked the centriole does not divide at the metaphase. The centrioles are double in the very early prophase, which may be simply a case of deferred division. There is no evidence that the astral rays reach the center to form a focal point; in fact, the evidence indicates that the centriole is an independent component. Random granules of the same size do not appear.

I appreciate the dangers of interpreting any structure apparent in a cell after having been fixed with a powerful chemical reagent and then stained with an artificial dye. In fact, the structure we see may not exist as such in the living cell.

It is equally obvious that it may be impossible to see in a living cell all of its components. A use of any observational method is not always sufficient. Further, it is almost axiomatic that staining reactions are very doubtful bases for chemical analysis of fixed objects. It is recognized that physical factors such as the amount of charge or density play as significant a part in color reaction as the actual chemical composition. According to some workers, for example, a basic dye has a tendency to precipitate upon the surfaces of more dense structures, while an acid penetrates more rapidly. I will not, therefore, attempt to attach a definite physico-chemical status to the centriole as found in *Ascaris megalocephala*. It might be suggested from a review of all the observations of many workers that it does have a specific identity, serving possibly as a locus of reaction as yet not fully determined. In *Ascaris* the component is apparent as a structure differing physically or chemically or both from the surrounding medium. It might be conceived that in other forms the structure might not be so compact and therefore not so easily demonstrable, but there is no convincing evidence of this.

SUMMARY

A review of the literature shows a centriole to be observed in the second cleavage of *Ascaris megalocephala*.

In the present work the centriole in the second cleavage shows an increasing affinity for the stain up to the metaphase and then a decreasing affinity, the shape is not constant, and the centriole apparently divides during the interkinetic period. The centriole also shows evidence of continuity from one cleavage to the next.

There is no evidence that it is the focal point of astral rays or a random granule.

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EXPLANATION OF PLATES

All figures were drawn at table level with the aid of an Abbé camera lucida. A Bausch & Lomb microscope was used, with a 10× ocular and a 1.9-mm. oil-immersion objective, giving a magnification of approximately 1580 diameters.

PLATE 1

EXPLANATION OF FIGURES

All the figures were drawn from material fixed in Gilson-Carnoy and stained with Heidenhain's iron haematoxylin.

1 Late telophase of first cleavage. The centrioles are small, but specific, located in a homogeneous unstained area (centrosome). No granules in the cytoplasm similar to centriole in staining capacity. Cytoplasm more vacuolated in middle region than at poles or at the line of cleavage.

2 Early prophase of AB cell in second cleavage, showing two centrioles. There is very little evidence of a centrosome or astral configuration.

3 Stage slightly later than figure 2. Note the increase in size of the centrioles.

4 Late prophase in AB cell. Centrioles have migrated nearly to opposite poles, but are not appreciably larger than the centrioles in figure 3.

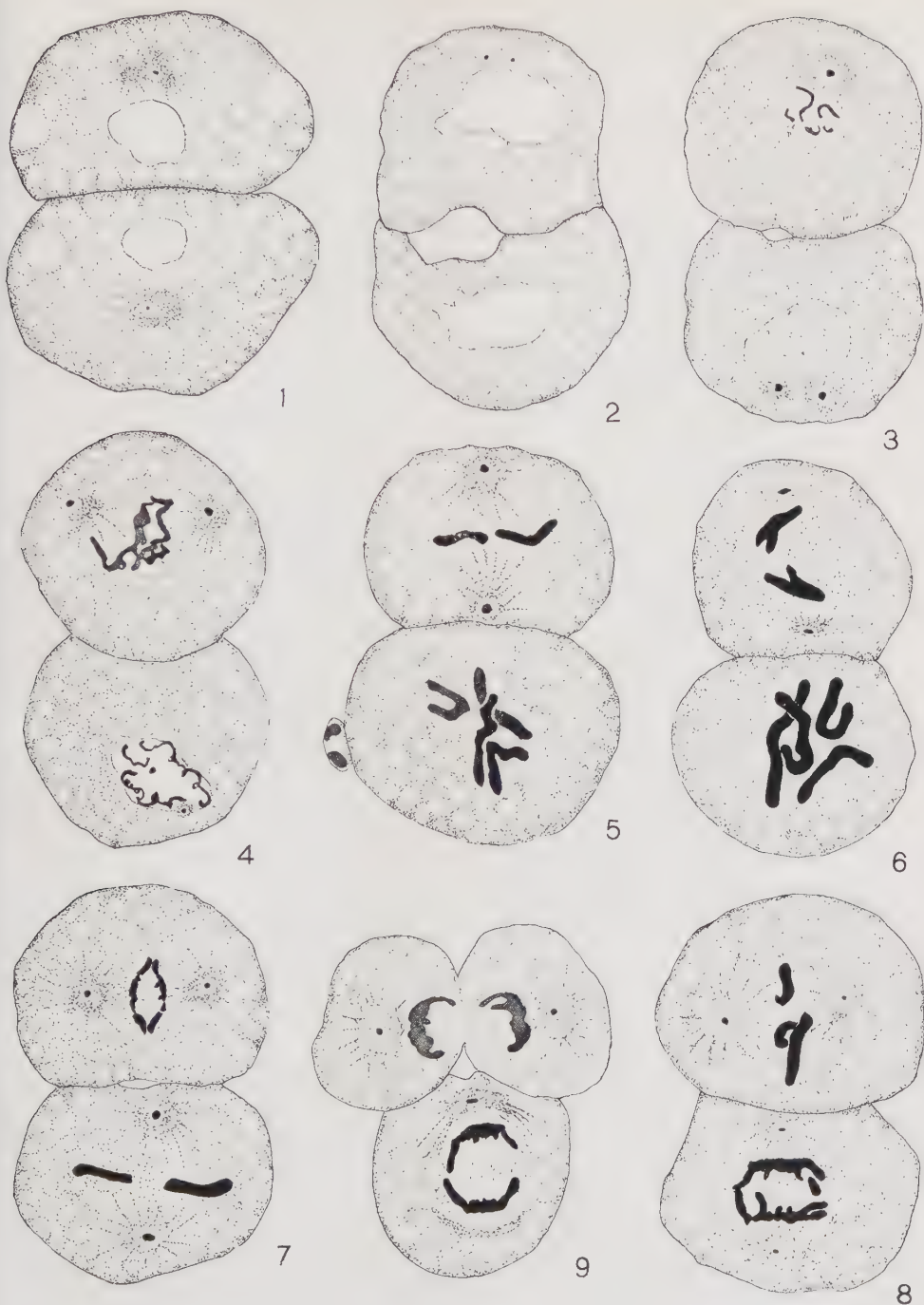
5 Metaphase of both AB and CD cells, AB in side view and CD in polar view. Note the irregular shape of the centrioles and the poorly defined centrosome and astral rays.

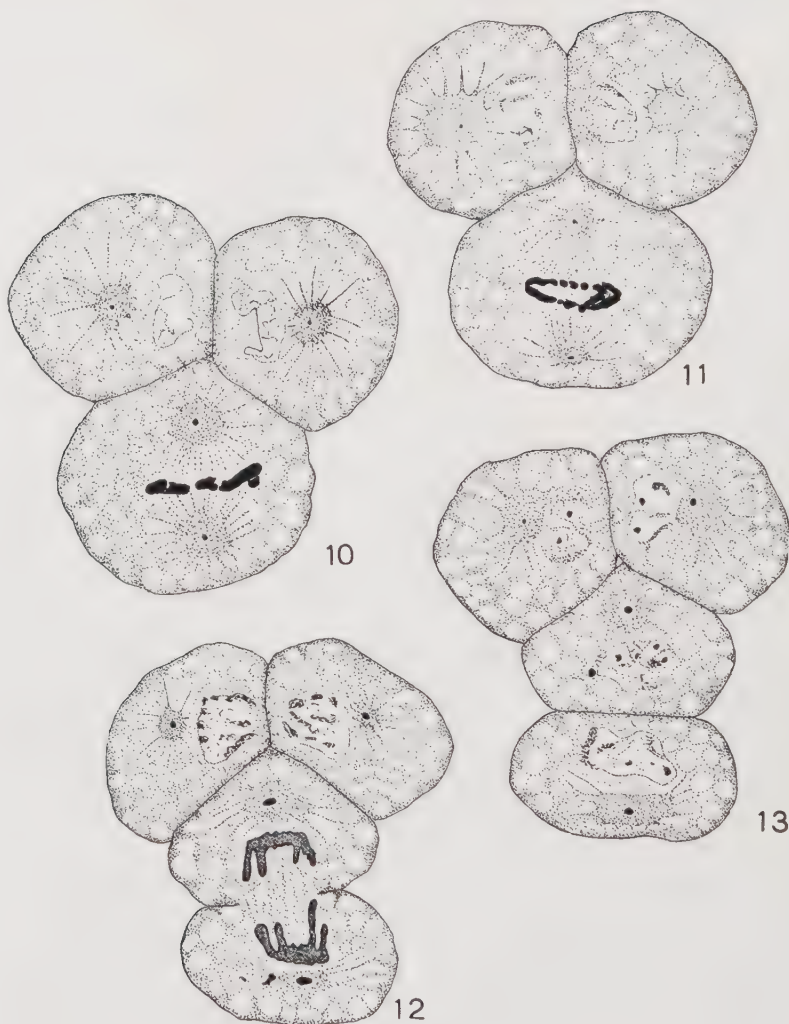
6 Anaphase of AB cell. Metaphase polar view of CD cell. Centrioles are elongate and irregular in shape.

7 Anaphase of AB cell and metaphase of CD cell. Four centrioles can be seen, all of different sizes and shapes. With the same degree of destaining, the centrioles of the metaphase are always larger than those of the anaphase.

8 Stage slightly later than figure 7. With the same degree of destaining, the anaphase centrioles are smaller and often somewhat elongate.

9 Early telophase in AB cell. Late anaphase in CD cell. The centriole in the CD cell is elongate and shows indication of a possible duality. In a later stage (telophase of AB cell) the elongation is no longer apparent.





10 Three-celled stage. No evidence of double centrioles in the A and B cells.
11 Three-celled stage. Destained nearly to point where centrioles would be no longer visible. No evidence of duality of centrioles. Note that the centrosome and astral rays are equally evident in all three cells.

12 T-celled stage only slightly destained. This figure is in contrast to figure 11 cut from the same section.

13 T-celled stage. Four centrioles showing when slide only slightly destained. Astral rays do not reach center of configuration. Centrioles not yet divided.

THE DIMINUTION OF THE GLYCOGEN STORE OF THE RABBIT PLACENTA DURING THE LAST THIRD OF PREGNANCY

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ONE COLOR PLATE (SIX FIGURES)

Glycogen was observed in the rabbit placenta by Claude Bernard in 1859, and in view of the rich glycogen deposits found early in pregnancy he wrote, "There exists in the placenta of mammals a function which up to the present time has remained unknown, and which coincides with the absence of the glycogenic function of the liver during the first period of embryonic life." Later investigators have studied the glycogen of the placenta from two chief standpoints, namely: 1) histological description of changes in distribution; and, 2) chemical determination of the quantities of glycogen involved.

In the rabbit placenta the various cells which are involved in the origin, storage, and release of glycogen have been carefully studied in histological observations by Maximow ('97) and especially by Chipman ('02) in a well-known monograph. In chemical analyses according to Pflüger's method, Lochhead and Cramer ('08) have further measured the changes in the glycogen content of the rabbit placenta at various stages of pregnancy. There is abundant evidence, therefore, from histological and chemical investigations, that the storage of glycogen is a conspicuous function of decidual cells of the rabbit placenta. The next question, namely, what part do these glycogen deposits play in the functioning of the placenta, would seem to involve, as Bernard implied, investigation of the glycogen stores of the foetus.

Such studies of the carbohydrate stores of the foetus have been carried out in this laboratory by Snyder and Hoskins ('28), and the attempt has been made to correlate the blood-sugar level of the foetus and the maternal animal and the glycogen content of the foetal body, foetal liver, placenta, and maternal liver at various stages of pregnancy. At present, then, it is possible to evaluate the glycogenic function of the decidual cell more adequately than has been possible previously, at least to the extent that new data are available concerning the blood-sugar and glycogen stores of the foetus in utero.

Before attempting to correlate the changes in glycogen storage of the placenta with the alterations of the glycogen of the foetus at various stages of pregnancy, it would be of definite advantage to obtain first-hand knowledge of the histological changes in the glycogen-storing cells of the placenta by direct observation. In the present study, therefore, the opportunity is taken to make such histological observations, and in view of the availability of the placentae of the identical animals upon which the blood-sugar and glycogen determinations have been made, the microscopic findings would seem to be of unusual interest.

Since the time of the earlier observers there have been advances, too, in histological technique and methods of chemical analysis, of which advantage may be taken in following changes in the distribution of glycogen. Also, the combined histological and chemical observations in a single litter, or even in one placenta, carried out in a single laboratory by one group of observers make possible closer correlations and controls. The stage of pregnancy in the rabbit may be determined to-day with accuracy, in view of the increased knowledge of the conditions associated with ovulation and implantation. The histological picture of the glycogen-storing cells of the placenta may thus be viewed under controlled conditions; the microscopical findings in a given placenta may be readily correlated with quantitative chemical determinations of the amount of glycogen in the foetal body, the foetal liver,

and the maternal liver, as well as the blood-sugar level of the foetal and maternal blood.

METHOD AND MATERIAL

In the present series of rabbits the placentae were obtained under conditions favorable for the preservation of glycogen. In a typical experiment the maternal animal was killed by a blow on the head and the uterus was quickly removed. The uterine wall opposite the site of implantation was incised and the placentae were carefully separated from their attachment. These were at once minced in 60 per cent potassium hydroxide for the chemical determination of the glycogen.

One placenta of the litter, however, was used for the histological study of glycogen. This placenta was divided into halves and one-half was fixed in absolute alcohol for histological examination, while the other half was kept as a control and the glycogen content was determined chemically. The tissue for histological examination was cut into thin slices and left in 200 cc. of absolute alcohol for about twelve hours. Blocks were embedded in paraffin and the sections mounted in the usual manner, except that warm alcohol was used instead of water. After passing through xylol and several alcohols, the sections were lightly stained with Ehrlich's hematoxylin. Best's carmine stain was then used. The sections were immersed in the carmine stain for about five minutes and differentiated in a solution of methyl alcohol and absolute ethyl alcohol. Counterstained sections showed no less glycogen than those stained only with carmine.

The stage of pregnancy was estimated from the time of mating, since ovulation has been observed to occur regularly about ten hours after coitus. The animals were reared in the laboratory for the most part, and were provided daily with an abundant diet consisting of cabbage, oats, alfalfa, salt, and water.

The histological findings are based upon the examination of the placentae of fifteen rabbits at various stages of pregnancy between the twenty-second and thirty-second day, i.e., the last ten days of pregnancy.

Chemical determinations of glycogen in the placentae of this series are available in unpublished records of work done in this laboratory by Snyder and Hoskins, who used Pflüger's method with certain modifications. Glycogen was estimated in forty-three placentae obtained from nine animals at various stages of pregnancy.

OBSERVATIONS

In the present series of placentae it becomes evident from histological study, as well as from the chemical determinations, that striking changes occur in the glycogen store of the organ during the last third of pregnancy. If the microscopical findings are first considered, it is found that at one extreme there are placentae which show extensive areas stained brilliantly red by Best's carmine (fig. 2), while in marked contrast there are other placentae which show much smaller deposits of red-stained material (fig. 3). The magnitude of the alterations in the glycogen deposits is illustrated in the plate in the contrast between figures 2 and 3.

Furthermore, if the stage of pregnancy at which the placenta was observed is also taken into consideration, it is found that the maximal store of glycogen in this series is present at an early stage; while on the other hand, at term, the glycogen deposit is at a minimum. Similarly, at stages of pregnancy intermediate between the twenty-second and thirty-second days, the red-stained areas of the glycogen store are in general intermediate in extent between the extremes illustrated in the plate (figs. 2 and 3). In a summary, such as table 1, of the details of the microscopical examination of the placentae in an attempt to estimate the extent of the areas stained brilliantly red with carmine, it is apparent that there is a gradual decrease in the glycogen store of the placenta as the age of the organ increases.

In the comparison of the amount of glycogen deposited in various placentae, it may be convenient to follow Chipman's description dividing the glycogen-containing cells into three chief zones: namely, the zone of separation, the perivascular

zone, and the intermediate zone. Thus in a placenta obtained from an animal on the twenty-third day, when glycogen is present in large amounts (fig. 2), the zone of separation is marked by a conspicuous deposit of glycogen which extends as a broad red band adjacent to the myometrium. In this region, at term, the separation of the placenta from the uterine wall occurs.

TABLE 1

Distribution of glycogen in the placenta of the rabbit. ++++ = maximal amount of glycogen. + = minimal amount of glycogen

STAGE OF PREGNANCY (DAYS)	RABBIT NO.	ENTIRE PLACENTA	GLYCOGEN SEEN MICROSCOPICALLY			GLYCOGEN DETERMINED CHEMICALLY	
			Zonal distribution			Total glycogen in one placenta (average), mg.	Glycogen per gram of placenta (average), mg.
			Zone of separation	Perivascular area	Intermediate zone		
22	76	++++	++++	+++	+++	98	14
22	275	+++	+++	++	++++	...	..
23	220	++++	++++	++++	+++	104	15
24	269	++++	+++	+++	+++	70	13
25	5	+++	+++	+++	+++	59	7
25	302	+++	+++	+++	+	...	..
26	282	+++	+++	+++	+	...	..
27	287	++	++	++	+	...	..
27	2	++	++	++	+	50	6
27	298	++	++	+	+	...	..
30	59	++	+	+	+	27	3
31	293	++	+	++	—	...	..
32	299	+	+	+	+	14	2
32	133	+	+	+	—	29	4
32	79	+	+	+	—	17	2

The perivascular zone includes the red-stained areas of glycogen which are seen encircling the large blood vessels and lying somewhat more remote from the myometrium (fig. 4). The margins of the perivascular glycogen deposits tend to be sharply defined.

There are also small red-stained cell clusters which border the trophoblastic ingrowth and comprise the intermediate zone. The region is marked by large cells densely packed with coarse carmine-stained material. Many of these cells

are of the giant multinuclear type (fig. 6). In the case of the glycogen-containing cells which lie close to the foetal placenta and sometimes appear in isolated clusters in the midst of the epithelium of the labyrinth, thus giving rise to some doubts as to their origin, there is no evidence in the present observations that they are not derived from maternal tissue.

In the placentae obtained at term (thirty-two days), it is evident that the red-stained areas have in large part disappeared (fig. 3). In order to avoid a possible diminution in the glycogen content of the placenta during the process of spontaneous extrusion at parturition, the animals were sacrificed by a blow on the head and the placentae quickly removed, just as was done at earlier stages.

Histological observations which show a marked decrease in the glycogen store of the placenta as term is approached receive additional support from the chemical determinations of the glycogen content according to analyses of Snyder and Hoskins. For instance, in chemical analysis of the second half of the placenta pictured in figure 2 and obtained on the twenty-third day of pregnancy, it is found that there are about 16 mg. of glycogen per gram of placenta. Five other placentae of this litter averaged 15 mg. of glycogen per gram of placenta, and each placenta had a total glycogen store of about 104 mg.

At the other extreme, in marked contrast to this large glycogen content, it is found on analysis of half of the placenta shown in figure 3, which was obtained at term, thirty-second day, that there are less than 3 mg. of glycogen per gram of placenta. Four other placentae of this litter also averaged less than 3 mg. of glycogen per gram of placenta, and each placenta had a total glycogen deposit of only 14 mg. In placentae which on chemical analysis or histological examination are found to have a glycogen store intermediate between these extremes, it is noted that the stage of pregnancy is likewise intermediate between the twenty-second and thirty-second days (table 1).

DISCUSSION

From the foregoing observations it is evident that there is a marked loss of glycogen in the placenta during the last third of pregnancy. There are extensive areas which are stained red with Best's carmine in the placenta at the twenty-third day of pregnancy (fig. 2), while at term (thirty-second day) the red-staining areas of glycogen are greatly diminished (fig. 3). In chemical determinations of the glycogen in the present series of placentae, Snyder and Hoskins have found that at the twenty-second day of pregnancy the placenta contains about 100 mg. of glycogen, while at term there remains about one-fourth of that amount, or 25 mg. Striking illustration is thus afforded of a measurable change of structure of the placenta as the duration of pregnancy advances, occurring even at a period so late as the last third of gestation.

A further morphological finding to be borne in mind in attempting to estimate the functional meaning of changes in structure of the placenta is the location of glycogen in the maternal portion of the placenta rather than in the foetal. Thus, the deposit of glycogen occurs in connective-tissue cells and is not evident in conspicuous amount in the epithelial tissue of foetal origin. This is in accord with observations of Chipman and of Lochhead and Cramer. Similarly, the huge giant cells of the obplacental region, which Sansom ('27) has traced back to a foetal origin, are found not to react to glycogen stains. In the rest of the body, of course, the great glycogen deposits are located in the muscle cells and in the epithelium of the liver. It has long been known that the amount of glycogen in the liver may vary within wide limits according to modification of food supply (MacLeod, '26, p. 140). For instance, after twenty-four hours' starvation, the glycogen of the liver may be nearly exhausted, while muscle glycogen may remain little changed. There is apparently a marked difference in the mechanism of control of glycogen storage and release in the case of liver epithelium, in contrast to muscle. In the placenta, therefore, where glycogen is

stored in still another type of cell, namely, connective tissue, it would seem especially difficult, in the absence of further observations, to understand the function of the glycogen store by analogy with the physiological rôle of glycogen in other organs.

SUMMARY

1. In the rabbit placenta there is a great diminution of the glycogen deposit during the last third of pregnancy.

2. Estimations of the magnitude of the change in sections stained with Best's carmine show that there is a loss of three-quarters of the glycogen store of the placenta.

3. Chemical determinations show that there is a decrease from 100 mg. of glycogen per placenta at twenty-two days to less than 25 mg. at term (thirty-two days).

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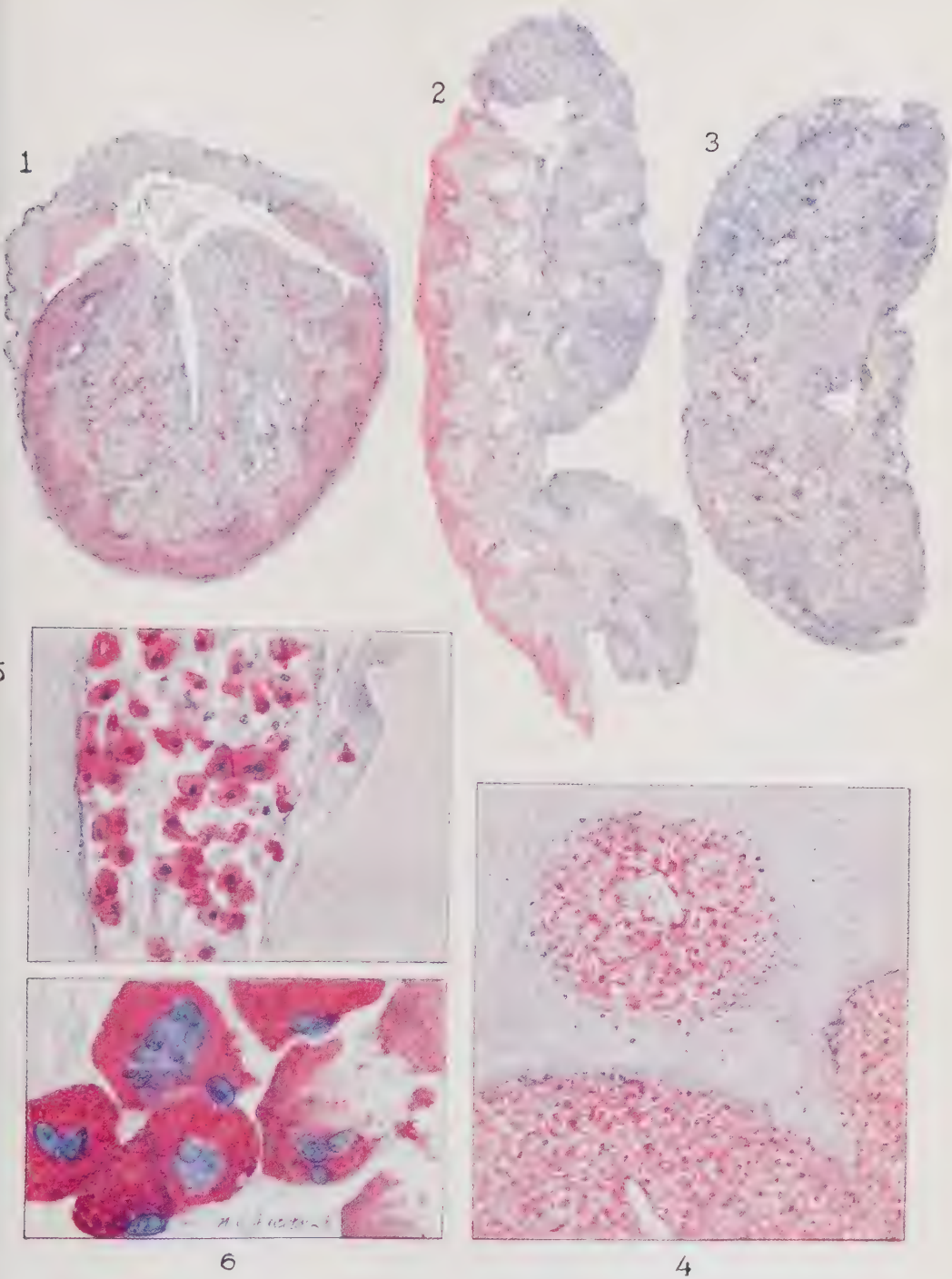
PLATE

PLATE 1

EXPLANATION OF FIGURES

The distribution of glycogen is shown in red as stained by Best's carmine method. The decrease in extent of red-stained areas in the placenta at term, figure 3, in contrast to the placentae obtained earlier in gestation, figures 1 and 3, illustrates the loss of glycogen as term is approached.

- 1 Fifteen-day placenta. $\times 4$. Rabbit 301.
- 2 Twenty-three-day placenta. $\times 4$. Rabbit 220.
- 3 Thirty-two-day placenta. Term. $\times 4$. Rabbit 299.
- 4 Twenty-three-day placenta. Perivascular zone. $\times 100$. Rabbit 220.
- 5 Twenty-three-day placenta. Structural changes of vessel wall. $\times 450$. Rabbit 220.
- 6 Twenty-three-day placenta. Multinucleate giant cells of intermediary zone. $\times 1000$. Rabbit 220.



VARIATIONS IN THE MITOCHONDRIA OF THE HEPATIC CELL IN RELATION TO ALTERATIONS OF THE GLYCOGEN-GLUCOSE EQUILIBRIUM

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FOUR TEXT FIGURES AND ONE DOUBLE HELIOTYPE PLATE (FOUR FIGURES)

The interpretations of the general functional significance of the mitochondria given by various investigators vary from denial of any grounds for conclusions of any kind to assignment of specific functions to these inclusions. In the case of any organ whose functions are varied, and this is true of any protoplasmic body, we anticipate difficulty in obtaining perfectly controlled conditions for experimental morphological work, in so far as the other functions are not perfectly understood. And the more varied the functions and greater our lack of knowledge concerning them, the greater is our source of error in supposedly controlled experimental work. In discussing the work that has been done on mitochondria this consideration is necessary, for otherwise we would be likely to regard the investigators in the field unjustly. This is especially true of the accounts which attempt to interpret the functional significance of the mitochondria of the liver, which the variety of the functions of this organ, reflecting the activity of its constituent cells, would lead us to expect.

Even a brief review of the vast literature dealing with this subject is unwarranted, as such résumés have recently been made (Noël, '23; Mann, '28). It is necessary, however, to mention the present status of the specific problem dealt with in this study, namely, the morphological accompaniments of the glycogen-glucose cycle. According to some of the earlier

investigators, exemplified by Arnold ('08), the mitochondria become hypertrophied in direct proportion to the amount of glycogen that is being formed. This hypertrophy was interpreted not as a true enlargement of the chondriosomes, but as distention resulting from the accumulation of glycogen within them. This idea has been opposed by other investigators, such as Bang and Sjövall ('16) and Noël ('23), who have shown that the distribution of glycogen does not conform at all to the distribution of chondriosomes, nor are the sizes of the particles comparable. From this Noël concludes that the glycogen cycle and the mitochondrial cycle are independent, and Mann states, "It is considered that the deposition of glycogen is accompanied by a change in the character of the protoplasm of the hepatic cell, but there is no demonstrable relation between such glycogen deposition and changes in the mitochondria" ('28, p. 226). Consideration of the observations of these workers reminds us that they were attempting to correlate mitochondrial morphology with static aspects of glycogen deposition. In the light of work that has been done on other types of cells, we would expect no observable relationship, since all successful attempts at correlation have compared mitochondrial morphology to constantly changing states relating to various phenomena, such as formation of secretory granules, and yolk, and the onset of pathological conditions.

In view of this, it seems desirable to investigate the morphology of the mitochondria of the hepatic cell at a time when the glycogen-glucose equilibrium is known to be disturbed. This will give an index of the glycogen cycle that is kinetic in character.

I wish to thank Prof. Irving Hardesty for the many courtesies extended to me as a visitor in the Department of Anatomy of Tulane University during the summer of 1930, where a portion of this work was done.

MATERIAL AND METHODS

The domestic cat was selected as the subject for this study. In all, sixteen have been used.

No food was given for a period of twenty-four hours and then they were disposed of in the following manner: 1) anaesthetized for a period of two hours, killed with a hammer, liver excised and fixed; 2) injected with adrenalin, liberated until marked muscular disturbance became evident, killed with a hammer, liver excised and fixed; 3) injected with insulin, liberated until death became imminent, killed with a hammer, liver excised and fixed; 4) killed with a hammer, liver excised and fixed. Four were killed at one time and the drugs administered so that they would all be killed within a few minutes.

In three of the series the blood sugar was not determined, but in the fourth it was, as a check upon the supposed effect of the above states on the sugar level of the blood. Benedict's method was employed.

Sections were cut in paraffin at 5μ and 2μ . Two stains were employed: Regaud's iron-alum-haematoxylin method and Benda's alizarin-crystal violet. Although Benda's gives a more specific and more beautiful stain, its erratic behavior at times makes the other method more desirable. Regaud's material has consequently been made the basis of most of the work, and Benda's has been used merely as a check on the other method.

OBSERVATIONS

Mitochondria of the hepatic cell at rest

The most extensive study of the morphology of the mitochondria of the liver is that of Noël ('23). This author divides the hepatic lobule into three zones, on the basis of mitochondrial variability, namely: 1) zone of permanent repose (perihepatic); 2) zone of permanent function (periportal); 3) the intervening variable zone. After a period of twenty-four hours' starvation, the mitochondria of these zones are, respectively, filamentous, spherical, short rods to

filaments. Noël's figures and descriptions are very clear-cut, so that one would have no occasion to doubt the accuracy of his observations. However, my own observations on the cat do not substantiate his on the mouse—a difference that may be due to the difference of material, although one would not expect such variability when both animals belong to the same class. In the cat the mitochondria in the cells surrounding the portal unit are more inclined to be filamentous than are those surrounding the central vein and are the last ones to assume a spherical shape when the liver becomes active, whereas those around the central vein are never long filaments, but vary from rods to short filaments. The intermediate zone exhibits a gradual transition from the one zone to the other; in fact, there is a very gradual transition all the way from the portal unit to the central vein (fig. 5). Thus, the distribution of the mitochondria of the hepatic lobule of the cat is the reverse of the condition described for the mouse. On one point, however, there is perfect agreement between Noël and myself—the relative numbers of mitochondria in the three zones. In the cells in the center of the lobule the mitochondria are so abundant that the view of them is very much confused except in extremely thin sections, while at the periphery the mitochondria are relatively scarce. There is a gradual transition from the one zone to the other, just as in the case of size and shape (fig. 5).

It is unfortunate that these observations cannot be harmonized with the extensive work by Noël, but since that cannot be done it is highly desirable to have this work extended both as to material and observers.

The filamentous type of mitochondria and the small spheres stain as homogeneous bodies not only with Regaud's method, but also with Benda's, that is, there is no differentiation of cortex and medulla, the chondriosomal material apparently being invariable throughout. The large spheres are also homogeneous after Regaud's procedure, but exhibit the mitochondrial reaction only in the cortex after Benda's method, the center remaining hyaline. This would suggest that per-

haps the greater part of the bulk added to effect this excessive growth of the chondriosomes is some storage product that is unrelated to true chondriosomal material or is of a vacuolar nature. It is these large seemingly hollow spheres that Noël has termed 'plastés.'

Mitochondria of the hepatic cell during a disturbance of the glycogen-glucose equilibrium

It is very difficult to alter the activity of an organ such as the liver with respect to only one phase of its functions. Thus, feeding an animal carbohydrate food not only disturbs the glycogen-glucose equilibrium in favor of glycogen, but also adds another factor—bile secretion. Consequently, it would be very difficult to ascertain which of these phases of its activity might be expressed in changes in its morphology. It would seem that more perfect control would be made possible by the administration of drugs which specifically affect the sugar level of the blood. In this case the greatest source of error would probably be the possibility of a direct effect of the drug upon the mitochondria, which might be unrelated to the sugar level. I am not inclined to give credence to this, because one would not expect insulin, adrenalin hydrochloride, and ether to have identically the same effect if their action were direct, and such is the result of their administration. The work of Soskin ('27) and of Olmsted and Simpson ('29) has clearly demonstrated that the sugar which is added to the blood in hyperglycemia produced by adrenalin and ether anaesthesia is derived from liver glycogen, rather than from any other portion of the body. Thus, we can feel sure that these two substances do disturb the glycogen-glucose cycle with respect to the liver.

I have utilized one means of producing hypoglycemia, namely, injection of insulin, and two means of effecting hyperglycemia, ether anaesthesia and injection of adrenalin. In no case was any attempt made to produce the maximum hypo- or hyperglycemia, but rather to kill the animal at a time when the sugar level was still increasing or falling. When the

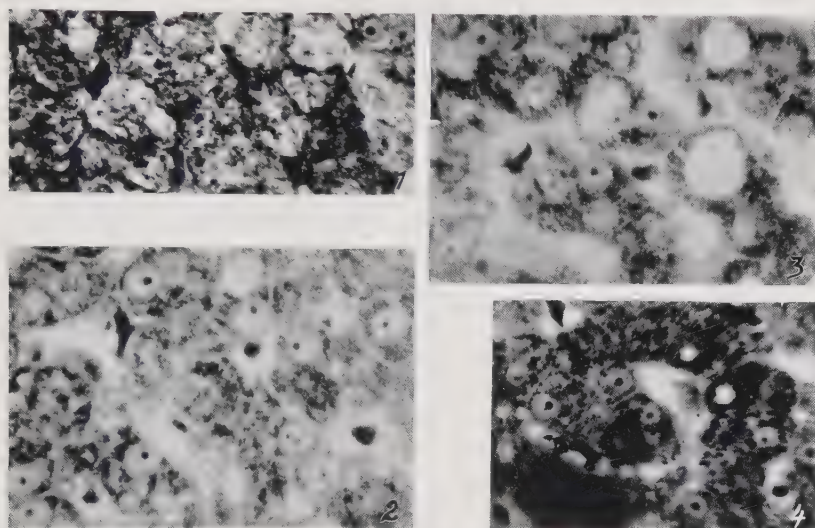
blood sugar was determined, it was found to be as follows: *a*) insulin—46 mg. per 100 cc. (cat 14); *b*) adrenalin—179 mg. per 100 cc. (cat 15); *c*) ether—219 mg. per 100 cc. (cat 16); *d*) normal—102 mg. per 100 cc. (cat 13). The illustrations are taken from these same four animals.

The morphology of the mitochondria of the liver cell is exactly the same in both hypo- and hyperglycemia and this condition is very materially different from the morphology of these constituents in a normal unfed animal. The response on the part of the mitochondria to these experimental conditions is, in a single phrase, a tendency toward enspherulation and hypertrophy. In the zone immediately surrounding the hepatic vein, those cells with a very definite cord-like arrangement, the mitochondria lose their rod-like character and become spherical. I have found many degrees of this process. Figures 2 and 6 are taken from the animal in which the mitochondria came nearest to retaining the rod-like character of those of the normal cell, and in this specimen much of the cylindrical nature of the rods has been lost. On the other hand, figures 3 and 8 represent the extreme of enspherulation. Here all are perfect spheres or nearly so. The tendency to become spherical is much less accentuated in the middle zone and still less in the periportal zone. Thus, in figures 2 and 6 the mitochondria near the portal unit are distinctly rod-like and it is only in extreme cases of mitochondrial response, such as figures 3 and 8, that they are spherical in this region also.

The second response on the part of the mitochondria, hypertrophy, is also more pronounced in the central portion of the lobule. In the less extreme cases the size is almost doubled, and in cases of extreme response, such as figure 3, the size is even more greatly enlarged. Although the hypertrophy is somewhat less in the periportal zone, it is, nevertheless, very noticeable.

In all cases of disturbance of the sugar level of the blood the mitochondria present a gradual transition, both as to size and shape and number, from the portal unit to the central

vein, just as in the normal unfed animal. The differences in the illustrations cannot be correlated with the substance used to alter the sugar level, as both ether and insulin apparently produced the greatest variation in other series, instead of adrenalin as in the group of four from which the drawings and photomicrographs were made.



Figs. 1 to 4 Photomicrographs of the central portion of hepatic lobules, taken from 2μ sections of Regaud material. Leitz apochromatic oil-immersion objective (1/12) and compensating ocular ($8\times$).

Fig. 1 From the liver of a normal cat, unfed for twenty-four hours (cat 13).

Fig. 2 From the liver of an unfed cat injected with insulin (cat 14).

Fig. 3 From the liver of an unfed cat injected with adrenalin (cat 15).

Fig. 4 From the liver of an unfed cat anaesthetized for two hours (cat 16).

The darkening effect of mitochondrial stains would lead one, after superficial examination, to conclude that hypo- and hyperglycemia cause an increase in the number of mitochondria. However, when one takes into consideration the increase in size of the chondriosomes, it seems likely that the closely packed condition in the experimental animals is not due to an increase in number, but merely the increase in size.

It is the large spherical mitochondria, such as those seen in figures 3 and 8, that fail to take Benda's stain except in the cortex. In the absence of any evidence reflecting upon the nature of the material constituting the core, we must recognize both the possibility that it is reserve material of some sort and also that the material of the core may be inconsequential and that the hollowing-out of the chondriosomes is an attempt to increase mitochondrial surface.

DISCUSSION

Validity of control measures

To obviate the suggestion that possibly this work was not perfectly controlled, it is well to state that it is not assumed that the control is absolute. The rate of metabolism as well as the amount of reserve food of various kinds differs in different animals. Therefore, the rate of conversion of glycogen into glucose differs in different animals, when they are subjected to the same period of starvation. Thus, we would not expect controls to be identical. The experimental animals, likewise, will differ to the same degree. In a word, perfect control is impossible in the case of any organ whose functions are so diversified as are those of the liver.

However, we can justifiably state that in these experiments three states of hepatic cells are being studied: *a*) from animals whose rate of conversion of glycogen into glucose is about normal; *b*) from animals whose rate of conversion of glycogen into glucose is faster than normal; and, *c*) from animals whose rate of conversion of glucose into glycogen is higher than normal.

Since the control is not absolute, we would anticipate that, if there is any relationship between mitochondrial morphology and carbohydrate metabolism, the mitochondria of the hepatic cell would not only differ in normal animals, but also in animals representing any specific sugar level, and this has been found to be true, as described above. Such variability does not detract at all from the significance of the observations recorded, because in no case does the tendency toward

enspherulation and hypertrophy of normal mitochondria exceed that of any experimental animal. In other words, the largest and most sphere-like mitochondria of a normal animal are smaller and less sphere-like than are those of the least modified case of hypo- or hyperglycemia.

Rôle of mitochondria

The extreme hypertrophy and enspherulation of the mitochondria of the hepatic cell during digestion that have been described by Noël ('23) are indicative of some relationship between the mitochondria and digestive activity. Noël concludes that the relationship is to bile secretion—a conclusion that is indicated, but is far from being clearly established. Since the mitochondrial response varies with the type of food, it might seem more likely that the relationship is to some other aspect of hepatic activity.

Contrary to earlier interpretations, it seems to me, the present account justifies the conclusion that there is some relationship between mitochondrial activity and carbohydrate metabolism. On the basis of this work alone it would be impossible to state whether mitochondrial substance acts as a catalytic agent affecting the conversion of glucose into glycogen, and vice versa, or is merely a marker for general metabolic activity. However, when we recall the work of Noël and attempt to harmonize the two accounts, each indicating a relationship to different aspects of hepatic function, it seems more likely that the variations in mitochondrial morphology are merely indicative of variations in the general metabolic activity of the structures involved. The variety of phenomena in other cell types which modify the configuration of mitochondria also lends support to this conclusion. These processes include such things as vitellogenesis (Kater, '28) and secretory activity (Moloy and Smith, '30), of which a discussion can be found in the affixed references.

SUMMARY

In the hepatic lobule of a cat that has been unfed for twenty-four hours the mitochondria are rod-like to filamentous, the shortest being found in the cells bordering the central vein and the most filamentous being located in the cells at the outer margin of the lobule.

The methods followed in disturbing the glycogen-glucose equilibrium were injection of insulin, of adrenalin hydrochloride, and ether anaesthesia. The animals were killed before the low point or high point of the blood-sugar level had been attained.

In all of the experimental animals the mitochondria exhibited a tendency toward enspherulation and hypertrophy, which was more emphasized in the cells bordering the central vein.

It is concluded that there is some relationship between the mitochondria of the hepatic cell and the glycogen-glucose equilibrium. It is further suggested that variations in mitochondrial morphology cannot be interpreted as indicative of specific functional changes, but only as an accompaniment of alterations of general metabolic activity.

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PLATE 1

EXPLANATION OF FIGURES

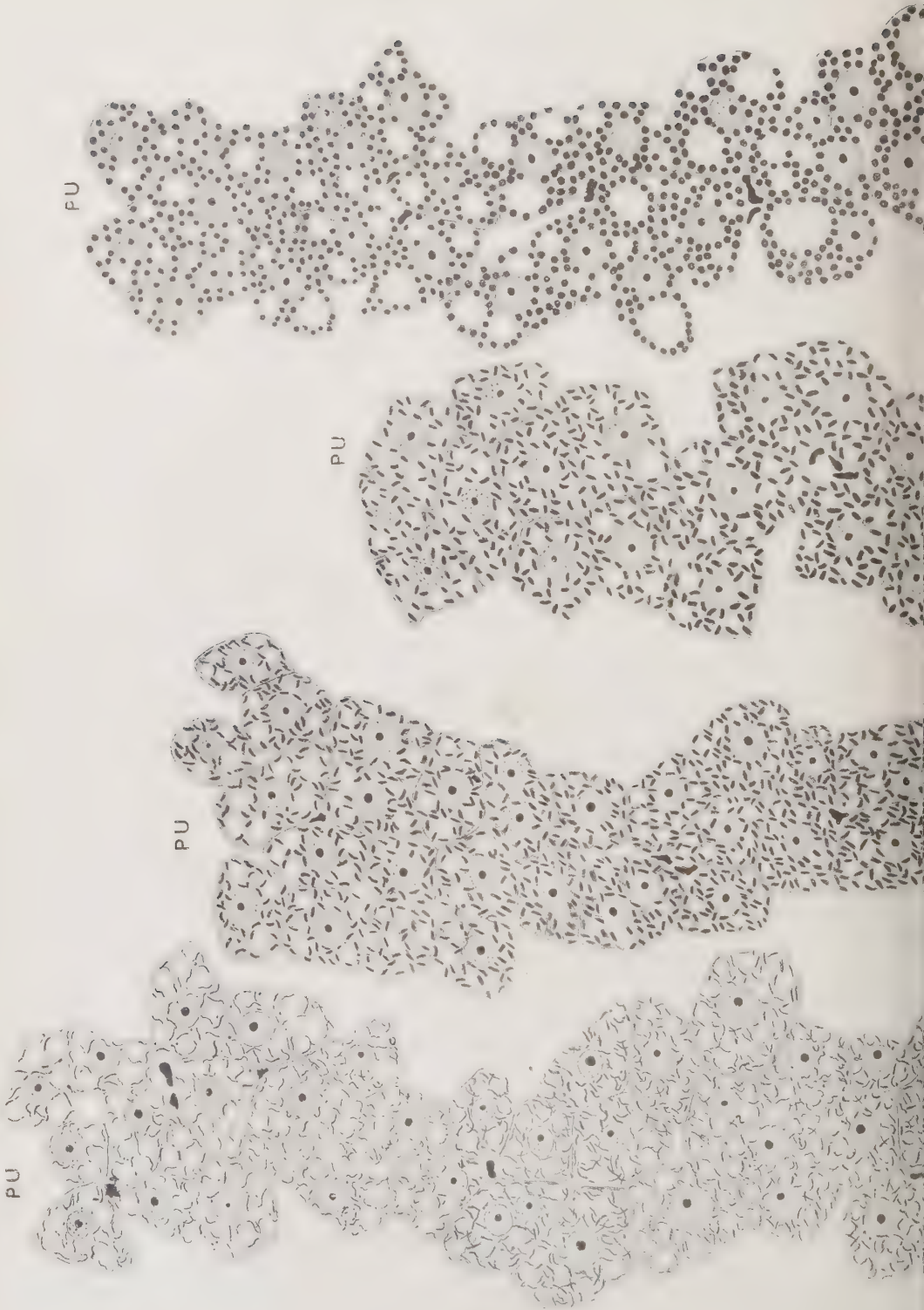
All figures were drawn from 5μ sections of liver, prepared according to the method of Regaud, under Leitz fluorite oil-immersion objective (1/16) with compensating oculars ($8\times$), with the aid of Abbé-model camera lucida. All figures include a sector of a lobule extending from the central vein to the portal unit. $\times 844$. *cv.*, region of the central vein; *pu.*, region of the portal unit.

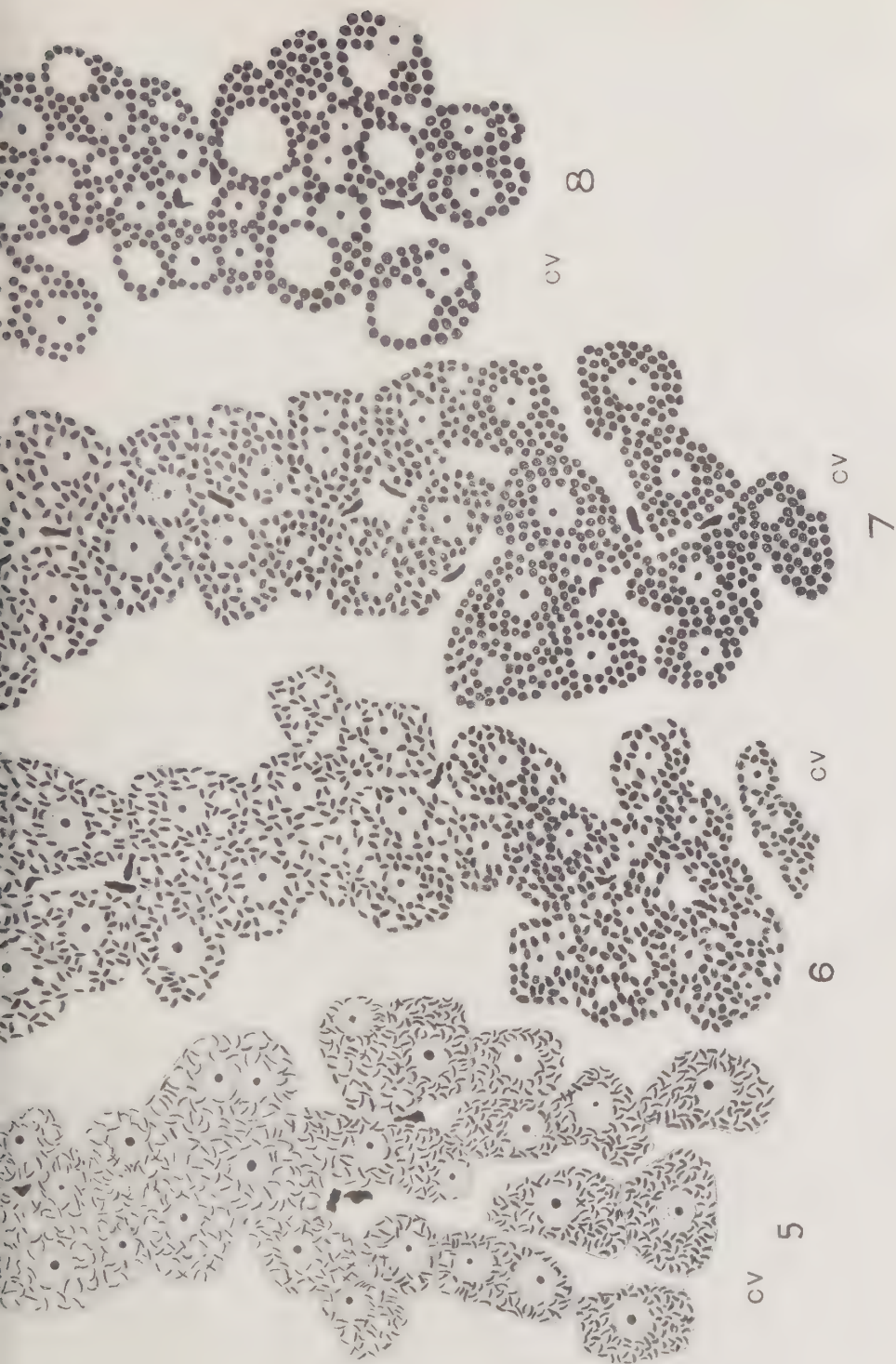
5 Sector of lobule of the liver of a cat that had been unfed for twenty-four hours (cat 13).

6 Sector of a lobule of the liver of a cat that had not been fed for twenty-four hours, then injected with insulin (cat 14).

7 Sector of a lobule of the liver of a cat that had not been fed for twenty-four hours; anaesthetized with ether for two hours (cat 16).

8 Sector of a lobule of the liver of a cat that had not been fed for twenty-four hours, then injected with adrenalin (cat 15).





“MANIFESTATION ALBERT BRACHET”

An international committee has been formed to establish a memorial to the late Albert Brachet, professor of anatomy and embryology in the University of Brussels, who died December 27, 1930.

Professor Brachet was noted for his work in the embryology of vertebrates and more particularly for his experiments on the early phases of development of the amphibian egg. His good sense, devotion, and unusual personal charm won for him a high place in the councils of science and education. American colleagues who met him on his extended tour of the United States two years ago will long cherish his memory.

The personnel of the committee is:

The Minister of Arts and Sciences of Belgium.

The Permanent Secretary of the Academy of Sciences.

The Permanent Secretary of the Academy of Medicine.

The President of the National Scientific Research Fund in Belgium.

The President of the Administrative Council, University of Brussels.

The Rector of the University of Liège.

The Rector of the University of Brussels.

The President of the Institute of Higher Studies in Brussels.

Professors

Bataillon, Montpellier

Boeke, Utrecht

Bordet, Brussels

Bouin, Strasbourg

Broman, Lund

Caullery, Paris

Collin, Nancy

Conklin, Princeton University

da Costa, Lisbon

de Winiwarter, Liège

Dubreuil, Bordeaux

Galpin, New York

Grégoire, Louvain

Gurwitsch, Moscow

Harrison, Yale University	Morgan, Pasadena
J. P. Hill, London	Rojas, Buenos-Aires
Ishikawa, Tokyo	Rouvière, Paris
Kostanecki, Cracow	Spemann, Freiburg
Latarjet, Lyon	van den Broek, Utrecht
Leboucq, Ghent	Vogt, Zürich
Levi, Turin	Weber, Geneva
F. R. Lillie, Chicago	Wilson, E. B., New York
Mohr, Oslo	

and the following who constitute the executive committee:

MM. L. Crismer, <i>President</i>	MM. Herlant
Dalcq	Lameere
Dustin	Marchal
Gerard, <i>Secretary</i>	Nolf
Govaerts	Philippson, <i>Treasurer</i>

The committee is asking for subscriptions to a fund to be used, 1) to found a triennial or quadrennial prize, to be awarded for the best work appearing during that period in the field of embryology, especially that of causal embryology, open to the entire world and to be awarded by the Belgian Academy of Sciences, and, 2) to place a memorial tablet to Professor Brachet in the entrance hall of the Institute of Anatomy in Brussels.

To subscribers of one hundred francs or more a reduced replica of the medallion will be given. Student subscribers will receive the replica for gifts of fifty francs or more.

Subscriptions should be sent directly to: M. Philippson, Banquiers, 44 rue de l'Industrie, Brussels, Belgium, and marked "Manifestation Brachet."

ROSS G. HARRISON.

INITIAL CHANGES IN THE TUNICS OF THE GALL BLADDER INDUCED BY EXPERIMENTAL LIGATION OF THE CYSTIC DUCT

E. A. HUNT, A. H. DAVIS, AND E. A. BOYDEN

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ONE PLATE (THREE FIGURES)

One of the unsolved problems of the extrahepatic biliary tract is the cause of pathological distention of the gall bladder with 'white bile' (hydrops vesicae felleae)—a problem that may be said to occupy a central position, not merely because of its clinical significance, but because it challenges our present-day knowledge of the histophysiology of this important organ. Among the questions thus raised is the extent to which resorption of bile occurs in the biliary reservoir, some authors having taken the advanced position that the pathological replacement of bile with a colorless fluid is evidence that the normal gall bladder resorbs the bile in toto.¹

The writer's attention was originally focused on the problem of hydrops by finding a cat with a normally contracted

¹In this connection it is desirable to dispose of the current misconception that seeks to place the storage and resorptive functions of the gall bladder in antithesis. Since much more bile is secreted every twenty-four hours than could possibly be stored in the biliary reservoir, it is obvious that the known concentration of bile by resorption of water and inorganic salts is an essential part of the storage mechanism. The real issue, therefore, is not whether the gall bladder is an organ of absorption, which everybody admits, but whether among other things it can normally resorb bile salts and bile pigment, as claimed by those who assert that the primary function of the gall bladder is "to return to the body (by absorption) the bile which is formed during the intervals between active digestion." In support of this theory (resorption of bile salts and bile pigments) no direct evidence has ever been advanced; opposed to it is the now well-substantiated fact that the gall bladder discharges much, if not all, its contents after meals (compare Boyden, E. A., 1928, *Anat. Rec.*, vol. 40, pp. 147-192).

gall bladder entirely devoid of contents, even of the usual film of inspissated bile. Also in this case the nodes at the intersection of the rugae were elevated above the level of the mucosa in the form of oedematous villi. Since the latter appeared to be moistened with mucus, it was thought that this condition might represent an early stage of hydrops.²

In the following preliminary study we have attempted to find out whether similar conditions could be produced in cats, experimentally, by ligating the cystic duct.

METHODS

Two ways of occluding the duct were tried, in the hope of discriminating between the effects of biliary stasis and interference with vascular drainage. In type A (fourteen animals) advantage was taken of the convoluted form of the cystic duct: a single loop being ligated in such a way as to avoid traumatizing adjacent structures. In type B (fourteen animals) that portion of the portal mesentery which contained the cystic duct, blood vessels, and lymphatics was enclosed in one ligature, but the latter was so tied as to occlude the duct but not stop the pulsation of the cystic artery. In approximately half the cases bile was withdrawn with a hypodermic needle from the fundus of the gall bladder and given to Doctor Davis for bacteriological examination; the tiny hole was then immediately closed by ligature. Needless to say, all operative procedures were performed under strictly sterile conditions. After the first operation, the cats were returned to their cages for from two to fifteen days, at the end of which period they were anaesthetized, the cystic bile again withdrawn aseptically, and samples of the liver secured for bacteriological culture. Then the gall bladder itself was removed, while the heart was still beating, and placed in fixatives. For each of the twenty-eight specimens the following reagents were employed, one for each piece of the gall bladder: Bouin, mercuric chloride, Ciaccio (followed by sudan III, for identification of cholesterol), and Flemming

² Boyden, E. A. 1928. Surg., Gyn. and Obst., vol. 46, fig. 5, p. 36.

(containing osmic acid for the detection of fats and lipoids). Tissues fixed in the first two reagents were stained with hematoxylin and counterstained in successive sections with eosin and with mucicarmine (the latter for detecting the presence of mucigenous granules and mucus). Altogether some 200 different sections, carefully prepared by Doctor Hunt, were available for study—usually not less than six from each gall bladder.

OBSERVATIONS

The first striking deduction from these experiments is that occlusion of the cystic duct, with consequent stasis, does not result in bacteriological infection of the contents of the gall bladder. Working exclusively on cats, we have found that the cystic bile was sterile in thirteen out of fourteen animals, notwithstanding the constant presence of *B. welchii* in the adjacent liver (table 1). The fourteenth animal (no. 81) contained a Gram-positive coccus not hitherto found by us in the liver. This confirms the findings of Toida,³ but is somewhat at variance with the recent work of Andrews⁴ who reports that "in 25 dogs all but three yielded positive cultures when the cystic or the common or the common and cystic ducts were tied." He therefore concludes that "in biliary stasis the gall bladder of the dog promptly becomes infected with the characteristic flora of the liver" (viz., *B. welchii*). From his preliminary report it is not apparent to what extent infection followed ligation of the cystic duct in his experiments, but it is obvious that obstruction of the common duct would induce changes in the liver that would not be duplicated by our experiments. At least, it would seem that in cats, ligation of the cystic duct does not induce bacterial infection of the cystic bile—that is, within the time needed to produce hydrops.

The second observation is that in all twenty-eight cases ligation of the cystic duct or mesentery is followed by some

³ Toida, R. 1920. Mitt. aus der I. Chir. Klinik, Kais. Univ. Kyushu, Bd. 5, S. 131-144.

⁴ Andrews, Edmund. 1931. Proc. Inst. Med. Chicago, vol. 8, p. 193.

TABLE 1
Bacterial examination of bile and liver

CAT	BILE FROM GALL BLADDER				LIVER SAMPLES ¹	DAYS AFTER LIGATION
	Before ligation of duct		After ligation of duct			
	Aerobically	Anaerobically ¹	Aerobically	Anaerobically ¹		
A-66			Negative, 7 days ¹	Negative, 30 days	Gram-positive cocci appearing in chains from 4 to 30. 48 hours. <i>B. welchii</i> , strep?	9
A-67			Negative, 20 days ¹	Negative, 20 days	<i>B. welchii</i> , 48 hours	9
A-68			Negative, 10 days ¹	Negative, 20 days	<i>B. welchii</i> , 48 hours	9
A-69	Negative, 10 days ¹	Negative, 37 days			<i>B. welchii</i> , 24 hours	
A-70	Negative, 15 days ¹	Negative, 37 days			<i>B. welchii</i> , 48 hours	
A-71	Negative, 10 days ¹	Negative, 37 days			<i>B. welchii</i> , 48 hours	
A-72	Negative, 10 days ¹	Negative, 37 days			<i>B. welchii</i> , 48 hours	
A-73	Negative, 12 days ¹	Negative, 20 days			<i>B. welchii</i> , 48 hours	
A-74	Negative, 10 days ¹	Negative, 20 days	Negative, 20 days ¹	Negative, 20 days	<i>B. welchii</i>	14
A-75	Negative, 10 days ¹	Negative, 30 days			<i>B. welchii</i> , 48 hours	
A-76	Negative, 16 days ²	Negative, 30 days	Negative, 17 days ²	Negative, 17 days	<i>B. welchii</i> , 48 hours	7
A-77	Negative, 16 days ²	Negative, 30 days	Negative, 17 days ²	Negative, 17 days	<i>B. welchii</i> , 48 hours	7
A-78	Negative, 17 days ²	Negative, 17 days	Negative, 9 days ²	Negative, 9 days	<i>B. welchii</i> , 48 hours	15
A-79	Negative, 16 days ²	Negative, 30 days	Negative, 9 days ²	Negative, 20 days	<i>B. welchii</i> , 48 hours	15
A-80	Negative, 19 days ²	Negative, 19 days	Negative, 17 days ²	Negative, 17 days	<i>B. welchii</i> , 48 hours	2
A-81	Negative, 10 days ²	Negative, 14 days	Gram-positive cocci (staphylococci?), 48 hours ²	Negative, 14 days	<i>B. welchii</i> , 24 hours	7
A-82	Negative, 19 days ²	Negative, 19 days	Negative, 17 days ²	Negative, 17 days	<i>B. welchii</i> , 48 hours	2
A-83	Negative, 21 days ²	Negative, 21 days	Negative, 13 days ²	Negative, 13 days	<i>B. welchii</i> , 48 hours	10
A-84	Negative, 19 days ²	Negative, 19 days	Negative, 12 days ²	Negative, 12 days	Negative, 12 days	7
A-85	Negative, 5 days ²	Negative, 12 days	Negative, 9 days ²	Negative, 10 days	<i>B. welchii</i> , 48 hours	3

¹ Cultured in meat infusion-dextrose broth.

² Cultured in meat infusion-dextrose-blood broth.

degree of cholecystitis, ranging from the mildest inflammatory reaction to hydropsy or necrosis. Since distention with 'white bile' must, in the last analysis, be referred to the mucous membrane of the gall bladder, the specimens are classified with reference to changes that occur in the tunica mucosa.

Group I (table 2, A) consists of fourteen cases in which the reaction is limited to moderate infiltration of leucocytes, chiefly neutrophiles. As this condition is not infrequently found in the gall bladder of unoperated cats, this group is classified as virtually normal.

Group II (table 2, B) consists of six cases in which the rugae are distinctly oedematous, but in which the gall bladder, following ligation of the duct and withdrawal of most of the bile, becomes fully collapsed. What remains of the fluid content is reduced to a greatly inspissated greenish black film or a colorless, glairy residuum.

Group III (table 2, C) consists of eight cases with oedematous rugae, but in this group the gall bladder is thick-walled and distended with a semiglairy fluid slightly tinged with green. With the exception of case A-50, the true condition of which was marked by the presence of bile pigment, these cases are typically hydropic.

For purposes of description the retrogressive changes in the mucosa of the gall bladder are divided into the following phases. Stage 1 represents simple oedema in which the capillaries of the rugae are distended and the connective-tissue cells of the tunica propria pressed apart by intercellular fluid. As a consequence the free ends of the rugae become uniformly swollen and project into the lumen in the form of bulging villi (fig. 1). Frequently the infiltrating leucocytes tend to congregate in the basal layer of the tunica propria.

Stage 2 may be characterized by the appearance of extensive accumulations of granular coagulum in the spaces of the tunica propria—evidently representing tissue fluid under pressure, since the surrounding connective-tissue cells are pressed back into limiting membranes. In this stage diapedesis of red blood cells first becomes noticeable.

TABLE 2
A. Mild inflammation; mucosa virtually normal

CAT	OPERATION	DAYS	GROSS APPEARANCE OF GALL BLADDER				HISTOLOGICAL APPEARANCE				
			At operation	Bile	At autopsy	Contents	Mucicarmine reaction		Mucosa	Muscularis	Peri-muscularis
A-43	A	7	Empty after meal Distended	Dark green residue	Collapsed	Viscid, green residue	Epithelium	Residue	Slight oedema, fat in epithelium	Normal	Normal
				Dark green	Moderately full	Viscid, green	Moderate	Moderate	Slight oedema, much cholesterol in epithelium, also fat	Hyperplasia	Some hyperplasia
A-45	A	12									
A-46	A	12	Distended	Dark green	?	Viscid, green	Strong	Strong	Slight oedema, cholesterol, some fat	Hyperplasia	Normal
A-52	A	14	Distended	Dark green	Half full	Viscid, green	Strong	Strong	Slight oedema, cholesterol, fat	Hyperplasia?	Normal
A-54	A	7	Distended	Dark green	Half full	Viscid, green	Strong	Strong	Normal	Normal	Oedema; marked infiltration
A-56	A	7	Distended	Dark green	Half full	Viscid, green	Strong	Strong	Slight oedema, cholesterol, much fat in epithelium	Slight hyperplasia	Normal
A-58	B	12	Moderately full	Dark green	Contracted	Viscid, green	Strong	Strong	Normal, some cholesterol	Normal	Local oedema
A-64	B	12	Moderately full	Dark green	Half full	Viscid, green	Slight	Strong	Epithelium filled with cholesterol, slight oedema	Normal	Oedema
A-68	B	9	Distended	Dark green	Half full	Viscid, green	Strong	Strong	Much cholesterol	Normal	Normal
A-76	B	7	Distended	Thick green, withdrawn	Collapsed	Viscid, green	Strong	Strong	Normal	Normal	Normal
A-77	B	7	Small, distended	Thick green, withdrawn	Collapsed	Viscid, green	Strong	Strong	Hyperplasia, infiltration in tunica propria	Infiltration	Oedema
A-78	A	15	Large, distended	Thick green, withdrawn	Collapsed	Viscid, green	Strong	Strong	Normal	Oedema	Oedema
A-82	B	2	Distended	Thick green, 1.5 cc. withdrawn	Nearly collapsed	Viscid, green	Strong	Strong	Normal	Normal	Oedema?
A-60	B	12	Distended	Amber	Collapsed, flattened	Viscid, white, hydropic	Slight	Slight	Epithelium loaded with fat and cholesterol in filtration, slight oedema	Normal	Normal

B. Mucosa oedematous, but gall bladder not distended with white bile

	B	10	Distended	Thick green, 1.7 cc. withdrawn	Collapsed, normal?	?	Strong	Strong	Stage 1	Normal	Marked oedema
A-83	A	2	Moderately full	Thick green, 1 cc. withdrawn	Collapsed, normal?	Viscid, green	Strong	Strong	Stage 1-2	Normal	Normal
A-79	A	15	Moderately full	Thick green, 1.5 cc. withdrawn	Collapsed, normal?	Viscid, green	Strong	Strong	Stage 1-2	Normal	Local oedema
A-55	B	7	Moderately full	Dark green	Collapsed, normal?	Black bile in mucus	Moderate	Moderate	Stage 1+, much cholesterol	Slight hyperplasia	Oedema?
A-81	B	7	Small, distended	Thick green, 0.7 cc. withdrawn	Collapsed, white	Viscid, white, bile-stained	Strong	Strong	Stage 2	Some oedema	Some oedema; many polymorphs
A-85	B	3	Distended	Amber, 1 cc. withdrawn	Collapsed	Viscid, white, bile-stained	Moderate	Moderate	Stage 1-2	Normal	Local oedema

C. Hydropic distention of gall bladder

	A	14	Distended	Dark green	Distended	Green bile, mucus and serous	Strong	Strong	Stage 1-2, much cholesterol and fat	Slight hyperplasia	Marked oedema
A-50	A	14	Distended	Dark green	Distended	Viscid, white, bile-stained; 0.3 cc. withdrawn	Strong	Strong	Stage 1-2, base of tunica propria dense with leucocytes	Some hyperplasia	Some oedema; marked infiltration
A-47	A	12	Distended	Dark green	Distended; thick, white	Viscid, white, bile-stained	Strong	Moderate	Stage 1-2, with flattened areas	Marked hyperplasia	Hyperplasia
A-51	A	14	Distended	Amber	Distended; thick, white	Viscid, white, bile-tinged	Strong	Strong	Stage 1-2	Marked hyperplasia	Marked hyperplasia
A-73	A	14	Distended	Thick green, 1.3 cc. withdrawn	Distended, hard	Viscid, white, bile-tinged; 2.5 cc. withdrawn	Scattered	Strong	Stage 1-4; marked distention in rugae; bile-stained	Normal	Hyperplasia
A-67	B	9	Moderately full	Dark green	Distended, translucent	Viscid, white, bile-tinged; 1.5 cc. withdrawn	Scattered	Strong	Stage 1-4; hemorrhagic and necrotic areas; pus in cavity; much cholesterol	Necrotic areas	Oedema
A-84	B	7	Distended	Thick green, 1.3 cc. withdrawn	Distended; thick, white	Viscid, white, 1 cc. withdrawn	None	None	Rugae hemorrhagic; denuded areas; pus in cavity; granulation tissue	Hyperplasia	Marked oedema
A-41	A	7	Distended	Dark green, pressed out before ligating	Distended; thick, white	Viscid, white, blood-stained	Scarce	Scarce	Necrosis; bile-stained; pus in cavity	Necrosis	Hyperplasia

In stage 3 (fig. 2) this diapedesis becomes so extensive as to amount to hemorrhage. At the same time the epithelium covering the villi begins to undergo retrograde changes, more marked at the apex and less noticeable toward the base of the rugae. At first these cells merely diminish in height, but as flattening proceeds they become atrophic and cease to stain with mucicarmine.

Stage 4 (fig. 3) is characterized by more or less sloughing of the epithelium, by diffusion of bile pigment into the tissues, by the necrosis of the mucosa, and by the appearance of pus in the lumen of the gall bladder.

As indicated in table 2, these changes may or may not be accompanied by hyperplasia of the muscle or perimuscular layers or by oedema of these tunics, but usually there is considerable infiltration of leucocytes.

DISCUSSION

From a study of these data it is evident that in half of the cases ligation of the cystic duct or mesentery produces a very mild form of cholecystitis, that in one-fourth it noticeably affects the mucosa, and that in another fourth it definitely causes hydropic distention. This preliminary study thus confirms the theory of Aschoff⁵ that all cases of hydrops must be caused by inflammation. The most significant feature of these experiments, however, is that hydrops has been produced in the presence of sterile bile. Although most investigators are agreed that obstruction of the cystic duct is a fundamental prerequisite, only Toida⁶ has succeeded in producing hydrops experimentally, and then only by injecting moderately virulent cultures of *B. coli* into the gall bladder after the cystic duct had been ligated. Our experiments would thus indicate that while hydrops may result from bacterial infection of an obstructed system, it is not infection that is the essential factor, but inflammation.

⁵ Aschoff und Baumeister. 1909. *Die Cholelithiasis*. Jena.

⁶ According to Galle and Vecchi, 1928, *Arch. Ital. di Chir.*, T. 21, pp. 298-308.

In considering the possible causes of inflammation following ligation of the cystic duct or mesentery in cats, only two alternatives seem to present themselves: 1) injuries due to interference with vascular drainage or blood supply; 2) the irritating action of stagnant bile. Opposed to the latter view is the fact that highly concentrated bile in large amounts may remain in contact with the mucosa for fifteen days after the cystic duct is tied (table 2, A) without causing marked oedema of the rugae. On the other hand, although hydrops sometimes occurs when most of the bile has been withdrawn from the gall bladder (table 2, C) marked oedema of the rugae frequently fails to result in hydrops in the absence of stagnant bile (compare table 2, B). Of course, hydrops might develop in a longer period of time; but it is believed that until chemical studies of stagnating bile have been made, biliary stasis must be considered as a contributory factor (see also p. 304).

In favor of the first hypothesis is the fact that some oedema is present, in one or more tunics, in almost all the specimens.⁷ Yet why should the rugae be virtually free from oedema in half of all cases? Granting that vascular interference may not always follow ligation, or that oedema may be present in that part of the gall bladder that was not sectioned, is it not possible that swelling of the rugae depends primarily upon interference with blood vessels, whereas swelling of the other coats may be due to either blood vessels or lymphatics or both? For it has recently been demonstrated that in cats the lymphatics are limited to the basal layer of the tunica propria and do not ascend into the rugae.⁸ To this may be added the fact previously noted that in most

⁷ The reason why it occurs no more frequently in type B than in type A (where only the duct has been ligated) is due, presumably, to the subsequent inflammation of the mesentery at the point where the cystic duct is tied, induced by pressure, rupture of the peritoneum, or presence of a foreign body. Obviously, it is almost impossible to regulate the amount of vascular obstruction, experimentally, in the cystic mesentery. The problem is further complicated by the occurrence in some cats of an extensive venous drainage from the wall of the gall bladder into the fossa vesicae felleae.

⁸ Winkenwerder, W. L. 1930. Bull. Johns Hopkins Hosp., vol. 46, pp. 272-295.

cats there are accessory cystic veins that would escape ligation when the mesentery is tied. Such anatomical considerations may also explain why different areas of the mucosa of a given specimen do not always present the same degree of inflammation.

This brings us to a consideration of the nature of 'white bile.' A glance at table 2 shows that in all except the necrotic areas the mucosal epithelium and contents of the gall bladder stain strongly with mucicarmine. Therefore, throughout the formative stages of hydrops, mucus is being continually produced. On the other hand, there is abundant evidence that the fluid of hydropsy is composed not only of mucus, but also of inflammatory exudates. Indeed, sections of the lumen reveal the existence of both viscid material and of coagula similar in appearance to that found in the tissue spaces of oedematous rugae (fig. 1). Presumably the serous fluid increases with the intensity of the reaction. Human cases of long standing (distended with yellow fluid) would presumably consist mostly of exudates.

But what becomes of the green bile? In early stages it is doubtless diluted and perhaps modified by the exudate; and in advanced stages (fig. 3), after the epithelium has sloughed, it can be seen diffusing into the tissues, where it is eventually taken up by the blood stream. The important work of Winkenwerder (l.c.) also suggests the possibility that the bile may be gradually disposed of before sloughing of the epithelium takes place. For he has shown that when the mucosa of the cat is subjected to the presence of ferrocyanide-citrate solution, certain epithelial cells become more permeable than others and let the dye through in large amounts. If this continues for more than forty minutes, even the nuclei of these cells stain with Prussian blue, indicating a lethal concentration in the cell. May not, therefore, the cells which cover the rugae become increasingly permeable as the underlying oedema progresses? Such absorption by dying cells, if it occurs, would undoubtedly hasten the processes of dissolution. Certainly there is nothing in this series of experiments

to substantiate the theory that the primary function of the normal gall bladder is to resorb all constituents of the bile. In fact, the evidence is quite to the contrary.⁹

CONCLUSIONS

1. Ligation of the cystic duct in cats, or tying of the cystic mesentery of these animals in such a way as to produce biliary stasis without interrupting arterial circulation, is always followed by some degree of cholecystitis. This ranges from mildest inflammation to hydropic distention of the gall bladder.

2. Such ligation is not followed by bacterial infection of the cystic bile.

3. Hydrops of the gall bladder may, therefore, occur in the absence of infection and must be attributed primarily to inflammation of the gall bladder, especially when diapedesis in the oedematous folds of the mucous membrane reaches the stage of hemorrhage.

4. It is believed that the evidence presented in these experiments favors the interpretation that hydrops is due to moderate interference with the blood supply and drainage of the cystic blood vessels, and that the irritating action of stagnant bile may be a contributory, though not essential, factor.

5. Disappearance of the bile pigment under such inflammatory conditions offers no proof that the primary function of the normal gall bladder is to resorb the bile in toto. In fact, inspissated bile containing both pigment and bile salts may be found in virtually normal gall bladders at least two weeks after ligation of the cystic duct.

⁹ See also Hunt and Boyden, *Proc. Soc. Exp. Biol. and Med.*, 1930, vol. 27, p. 645.

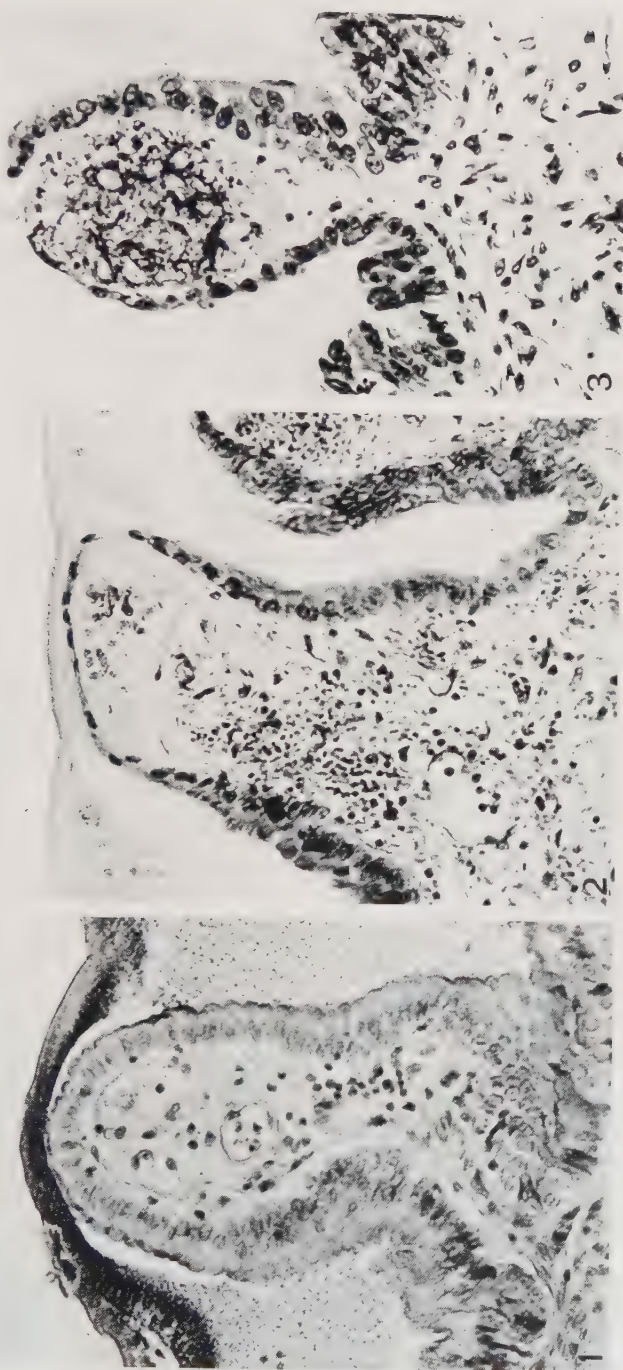
PLATE 1

EXPLANATION OF FIGURES

- 1 Fold of mucous membrane of gall bladder showing first stage in oedema. Fourteen days after ligation of cystic duct (cat A-50). Fixed in Bouin's fluid; stained with hematoxylin and mucicarmine. $\times 320$. Note mucus in epithelial cells (black areas corresponding in position to cuticula), dark-staining film of mucus covering ruga, coagulated exudate between epithelium and film of mucus, oedematous tunica propria, infiltration of leucocytes, and distended capillaries containing neutrophiles. (For second stage see fig. 5, Boyden, 1928, Surg., Gyn. and Obst., vol. 46, p. 36).
- 2 Fold of mucosa showing third stage. Fourteen days after ligation of cystic duct (cat A-73). Fixed in mercuric chloride; stained with hematoxylin and eosin. $\times 320$. Note extensive diapedesis of red corpuscles in tunica propria, flattening of apical epithelium, accumulation of exudate between epithelium and tunica propria.
- 3 Fold of mucosa showing fourth stage. From another part of the same gall bladder (cat A-73). Fixed in mercuric chloride; stained with hematoxylin and eosin. $\times 320$. Note sloughing of epithelium, diffusion of bile pigment in tunica propria.

CHANGES IN THE TUNICS OF THE GALL BLADDER
E. A. HUNT, A. H. DAVIS, AND E. A. BOYDEN

PLATE 1





A CYTOLOGICAL STUDY OF THE SPINAL-GANGLION
CELLS OF THE RAT, WITH SPECIAL REFERENCE
TO THE GOLGI APPARATUS, VACUOME, CANALIC-
ULAR APPARATUS (SAFTKANÄLCHEN), MITO-
CHONDRIA, CLEAR CANALS OF PENFIELD, AND
NISSL BODIES¹

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TWO PLATES (FIFTEEN FIGURES)

INTRODUCTION

The purpose of the present study has been to repeat, so far as it is feasibly possible in the spinal-ganglion cells, the classical findings of Golgi ('98), Holmgren ('14), Cowdry ('12, '14), Penfield ('21), Covell and Scott ('28), and others, in the hope of throwing some light on the present confusion that exists in the interpretation of cytoplasmic structures. The material selected for this study is similar to that used by Golgi in his original experiments on the 'apparato reticulare interno,' by Holmgren on the trophospongium, Cowdry on mitochondria and canalicular apparatus, Covell and Scott on the vacuome, Nissl and Held on the Nissl substance (chromatic substance, tigroid bodies, etc.), and Penfield on the simultaneous existence in the same cell of the Golgi apparatus and clear canals. The importance of using the spinal-ganglion cell in such an experiment has recently been pointed out by Covell and Scott ('28) and by Hibbard ('30), who call attention to the fact that the nerve cell constitutes the type apparatus, since it formed the chief material upon which Golgi's observations were originally made. In justice to

¹ Demonstrated before the American Society of Zoölogists, Cleveland, Ohio, 1930.

Golgi, however, it should be noted that his description of the 'apparato reticulare interno' is not 'a specific observation in nerve cells,' as has been recently stated by Hibbard ('30).

Since I have previously pointed out some of the current problems involved in the study of the cytoplasm ('30 a, b), they need not be repeated here. For a review of the general subject of cytoplasmic components the reader is referred to the works of Coghill ('04), Holmgren ('04), Golgi ('09), Duesberg ('14), Pappenheimer ('16), Penfield ('21), Cowdry ('24), Bowen ('27), Parat ('28), Gatenby ('30), Alexenko ('30), Ludford ('30), and others.

To anticipate the conclusions of this investigation, it may be said that the Golgi apparatus, mitochondria, vacuome, and Nissl bodies are discrete in the spinal-ganglion cells of the rat. The 'Saftkanälchen' of Holmgren are thought to represent the negative image of the Golgi apparatus. The clear canals of Penfield ('21) are probably quite distinct from the Saftkanälchen of Holmgren. No evidence was found in the spinal-ganglion cells which could possibly be interpreted to mean that the apparatus of Golgi is formed by the coalescence of vacuoles and granules that are specifically stained by neutral red, as held by Parat ('28), nor is there the slightest evidence to support his most recent view that the Golgi apparatus is formed from the mitochondria and is in reality a network of 'active' chondriosomes (Parat, '28, '29).

It is a pleasure to record here my deep appreciation to my colleague, Prof. Robert L. King, for many helpful suggestions given me during the progress of this work. I am likewise greatly indebted to Mrs. E. Powers for her painstaking care in making the figures.

MATERIAL AND METHODS

The material for this study consists of spinal-ganglion cells of the rat (*Mus norvegicus albinus*). All the animals were approximately the same age (eight to ten weeks) when sacrificed. In some cases they were killed by exposure to the

fumes of chloroform and ether, in others by a quick blow on the head. No cytological differences could be attributed to either method. The rats were reared on a well-balanced diet and housed in a vivarium maintained at a fairly constant temperature.

To demonstrate the Golgi apparatus the methods of Mann-Kopsch (Weigl) as modified by Ludford ('26) were generally followed. However, the time of exposure of the tissue to the 2 per cent osmic acid was somewhat varied, depending on the desired degree of impregnation. To determine the degree of impregnation, Nassonov's method of removing pieces of the impregnating tissue from time to time and crushing them in glycerin was employed. Another method which was used to advantage consisted in removing tissue from the 2 per cent osmic acid, dehydrating and clearing it in the usual manner, and mounting it in balsam; then by a gentle rotary movement of the cover-glass the cells were dissociated to the desired degree. This method has certain advantages in a study of the spinal-ganglion cells, in that it constitutes a permanent preparation and also furnishes a method by which it is possible to study the cells in toto, which is highly desirable as a supplementary method to sections in determining the degree of reticulation of the Golgi apparatus. Slides of sections that were overblackened with osmic acid were frequently bleached in hydrogen peroxide or exposed for long intervals to the oil of turpentine.

The uranium nitrate-silver method of Cajal was found useful in the demonstration of the Golgi apparatus. It impregnated the Golgi apparatus in every way comparable to the method of Mann-Kopsch (Weigl).

To demonstrate the canalicular apparatus of Cowdry ('12) and the Saftkanälchen of Holmgren ('14) the formol-potassium-bichromate method of Regaud ('10) was employed. Certain clear spaces which are interpreted as the clear canals of Penfield ('21) appear in some preparations following the Mann-Kopsch (Weigl) technique. These clear spaces are probably quite distinct from the Saftkanälchen of Holmgren and the canalicular apparatus of Cowdry.

The vacuome of Parat ('28) and Covell and Scott ('28) may be caused to appear by the injection intraperitoneally or subcutaneously of a 1 per cent solution of neutral red in normal saline. For the demonstration of the vacuome and the Golgi apparatus in the same cell the double method of injecting neutral red first and subsequently exposing the tissue to modified Mann-Kopsch (Weigl) technique, as described by Beams ('30 a), was followed. Subsequent dehydration and clearing were accomplished in a manner similar to that used in the Mann-Kopsch (Weigl) technique alone.

To disclose the Nissl bodies (tigroid bodies, chromatic substance) the alcoholic methylene-blue method was utilized; also, the method recommended by Delaney ('27) was found to give excellent results. To test the theory that the Nissl substance (chromatic substance) is of chromatin nature the 'Nuclealreaktion' of Feulgen was employed. This method was used in accordance with the directions given by Ludford ('28).

For the demonstration of the mitochondria the vital method of Janus green B, as recommended by Cowdry ('18), and the permanent techniques of Regaud ('10) and Bensley ('11) were employed.

GOLGI APPARATUS

The Golgi apparatus was first described in the spinal-ganglion cells by Golgi in 1898 by use of his well-known black reaction (*reazione nera*). Golgi considered the internal reticular apparatus as a new structure that is present in the cytoplasm of the spinal-ganglion cells in a position between the nucleus and the cell surface. He suggested that the structure might be found in other cells, and in 1909 he published his results on a study of the epithelial cells of the intestinal tract and reviewed the work of his students and others on the Golgi apparatus in a wide variety of cells. The Golgi apparatus has been seen in the spinal-ganglion cells by many investigators, among whom might be mentioned Kopsch ('02), von Bergen ('04), Cowdry ('12), Cajal ('14), Penfield ('21), and Alexenko ('30).

The Golgi apparatus in the spinal-ganglion cells of the rat is essentially like those described by many other investigators in the spinal-ganglion cells of other mammals. It appears typically as a paranuclear network of fairly smooth osmophilic canaliculi (fig. 1). The anastomosing strands appear to possess a somewhat variable girth and may display a certain degree of incompleteness. As far as can be determined, no definite pattern underlies the arrangement of the strands of reticulation and all variations of their form and position may be found. It is apparent that the Golgi apparatus does not penetrate the surface of the cell, although it is true that the filaments may in certain cases approach its surface. In like manner the Golgi apparatus has never been observed to encroach on the nucleus, nor has evidence been found which would indicate that the Golgi substance is derived from the nucleus in a manner similar to that recently suggested by Honda ('29) for the acinar cells of the submaxillary glands of the rat.

Some question has arisen recently regarding the interpretation of Golgi that the internal reticular apparatus is a closed network (Esterman and Gitlity, '27). In many cells of the spinal ganglion I have observed that the reticulation appears to be complete as far as it can be carefully followed into the depth of the cell (fig. 2). This is particularly true of cells of medium and small size.² However, this is not the case in all the cells, and many can be found which display only an incomplete reticulum, so that long or short cords or fragments of the reticulation are found in the same general position in the cells as that usually occupied by the closed reticulum (fig. 3). All gradations between these two conditions may be found. In still other cases I have observed the complete dissolution of the Golgi apparatus into granules and vacuoles (fig. 4). This condition is seemingly an artifact caused by the unsuccessful fixation of the Golgi apparatus by

²It seems probable that the Golgi material is present in many of the cells of invertebrates in the form of discrete bodies (dictyosomes). This is particularly true of the ganglion cells of insects which will be reported in detail in a forthcoming paper.

the technique employed, leading to the disintegration of its reticular structure. Similar action of the Golgi apparatus has been shown to take place following the exposure of the animal to various experimental conditions (Cowdry, '24; Ma and Tso, '30). It is obviously a very difficult task to demonstrate actually in these preparations whether the Golgi apparatus normally exists as a complete network in the living cell (its occasionally fragmentary appearance being due to the technique employed) or whether it is actually present in some cells as an incomplete reticulum. However, its behavior in gland cells during secretion indicates that it often breaks up, although subsequently the same apparatus may presumably resume its reticular form (Cramer and Ludford, '26).

I have not been able to detect vacuoles or granules (neutral red) in the substance of the Golgi filament as recently depicted by Alexenko ('29, '30). It is true that many of the granules lie in juxtaposition to the Golgi apparatus, but I am not sure whether this implies a physiological association or is simply a fortuitous position. Furthermore, it is of interest to note that in preparations which show clearly the Nissl bodies the Golgi material is usually present as isolated filaments (fig. 3). It must be pointed out, however, that the Golgi apparatus may exist in a similar fashion in cells in which the Nissl bodies are not apparent.

My interpretation of figures such as figure 4 is at variance with those of Misch ('03) and von Bergen ('04), who state that the vacuoles fuse to form the apparatus of Golgi. But, quite to the contrary, it seems to the writer that this condition represents the breaking up (dissolution) of the Golgi apparatus. A similar interpretation is suggested as regards Owens and Bensley's ('29) figure 3, which they have proposed to explain on the basis of surface impregnation of mitochondria. It seems that the primary impregnation of the Golgi apparatus causes it to appear as a thin incomplete filament which becomes more robust as the impregnation progresses up to a certain stage. Excess impregnation causes a fuzzy irregular contour of the osmiophilic strands of the Golgi

apparatus. However, this condition can often be removed by prolonged treatment with turpentine, which leaves only the smooth osmiophilic filaments.

There sometimes appear on the spinal-ganglion cells following the osmic-acid technique small dark granules. These structures are much smaller than the neutral-red bodies and more numerous than the 'lipoid globules' of Cowdry. They probably represent either diffuse lipoid granules or are induced structures.

I have been unsuccessful in finding any indication of such a chromophobic portion of the Golgi apparatus as that which is so distinctly shown in certain of the invertebrate cells. The dark osmiophilic filaments appear smooth and definitely demarcated from the surrounding cytoplasm. This suggests that the Golgi apparatus is probably bounded by a surface film at its interface with the cytoplasm. Such a membrane has not, however, been definitely demonstrated in this study.

If one compares the condition here described for the Golgi apparatus in the spinal-ganglion cells with the photomicrographs of the trophospongium in spinal-ganglion cells (Holmgren, '14), it seems obvious that the two are identical. They both possess the same form and distribution in the cell and both react to prolonged treatment with osmic acid in a similar manner. However, the connection between the network and peripheral 'trophocytes,' invariably found by Holmgren, is still unexplained, since the Golgi apparatus has been found to have no connection with the cell periphery.

CANALICULAR APPARATUS (SAFTKANÄLCHEN OF HOLMGREN)

Shortly after Golgi's ('98) original communication of the discovery of the internal reticular apparatus before the medical society of Pavia, Holmgren ('00), Nelis ('99), and others found a system of clear spaces in the cell, 'Saftkanälchen,' later called 'Kanälchen,' which Holmgren considered as a liquefaction of the trophospongium. The apparatus of Golgi is considered by Holmgren to be the intracellular portion of the trophospongium. Cajal ('08) reached the conclusion that

the apparatus of Golgi and the Saftkanälchen or juice canals of Holmgren were identical and proposed to call them 'conduits de Golgi-Holmgren.' von Bergen ('04), Bensley ('11), Duesberg ('20), Saguchi ('20), Cowdry ('24), Bowen ('28), and others have accepted the interpretation that the 'Kanälchen' of Holmgren comprise the negative image of the apparatus of Golgi. However, as we shall see later, Penfield ('21) has recently brought forth evidence which in his opinion seriously questions this interpretation.

To avoid confusion, I will use the term canalicular apparatus to indicate the Saftkanälchen of Holmgren and the type-I canals of von Bergen. In the spinal-ganglion cells this apparatus seems to have the same general topographical position in the cells as that occupied by the classical Golgi apparatus (fig. 5). The clear canal-like spaces are about the same caliber as the apparatus of Golgi. They are sharply delimited from the surrounding cytoplasm, which suggests that in the living cell some form of limiting membrane must intervene between the two bodies of cellular substance. From all appearances the canalicular apparatus is simply the mold of which the Golgi apparatus forms the cast. In other words, the Golgi substance has been dissolved away by the technique employed, leaving only the clear canaliculi. This view finds support in the fact that the canalicular apparatus is wholly intracellular and has no direct communication with the nucleus or extracellular structures. Furthermore, the clear 'canals,' like the Golgi apparatus, may be discontinuous or possess a more or less complete reticulum which is arranged in a paranuclear fashion between the nucleus and the periphery of the cell.

The contours of the canaliculi are oval and do not show evidence of shrinkage spaces as might be the case with the clear canals of Penfield later to be described. Furthermore, the interpretation that the canalicular apparatus in the spinal-ganglion cells is merely the negative image of the Golgi apparatus is supported by the work of von Bergen, who found in certain cases only shreds of the blackened Golgi substance

surrounded by clear spaces. Bergen interpreted this to mean that the Golgi apparatus had been partially impregnated, therefore representing a transition between the canalicular apparatus and the apparatus of Golgi. These observations find strong support in the work of Saguchi ('20), who studied the acinar cells of the pancreas by means of Flemming fixative and iron-hematoxylin stain.

The mitochondria are often present in preparations which show clearly the canalicular apparatus (fig. 5). This suggests that the chemical nature of the Golgi apparatus is considerably different from that of the mitochondria. Moreover, no transitional stages such as urged by Parat ('28, '29) are to be found between the mitochondria and the canalicular apparatus.

PENFIELD CANALS OR TYPE-II CANALS OF VON BERGEN

Long ago, von Bergen ('04) described certain clear spaces (type-II canals) in the spinal-ganglion cells which he suggested were probably artifacts and distinct from the negative image of the apparatus of Golgi (type-I canals). More recently, Penfield ('21) and Da Fano ('21) have found a system of clear spaces in nerve cells which are in many ways comparable to the type-II canals described by Bergen. They hold that the clear spaces described by them are the same as Holmgren's trophospongial canals. It will be recalled here that Holmgren explains his *Saftkanälchen* on the basis of a liquefaction of the trophospongium and that he contends that his trophospongium is identical with the apparatus of Golgi and the *Binnennetz* of Kopsch.

In some of my preparations following the technique of Mann-Kopsch (Weigl) I have found what appears to be the clear canals depicted by Penfield (figs. 6 and 7). These lacunae are found throughout the cytoplasm, with a slight concentration, perhaps, at a region near the periphery of the cell. These clear spaces often appear as if they might be directly connected with the surface of the cell. They have never been observed to form a continuous network and often

present lacunae of considerable variation in caliber. The contours of these clear spaces have a tendency in many cases to be strikingly irregular and often of angular form.

No observer who has studied these clear spaces in my preparations has failed to be struck by the marked difference in finer details between them and the canalicular apparatus of Cowdry and the Saftkanälchen of Holmgren. They are not the unstained ghosts of the Golgi apparatus, as Penfield maintains, but they represent a second system of clear spaces in some of the spinal-ganglion cells which are quite distinct from the negative image of the apparatus of Golgi. They can be demonstrated simultaneously in the cell with the vacuome and apparatus of Golgi (figs. 6 and 7). They are never stained by neutral red in the living condition or blackened by osmic acid. Consequently, it seems to the writer that Penfield is perfectly right in his description of these clear spaces, but that he has gone too far in his interpretation that they have been mistaken by others as the negative image of the apparatus of Golgi.

An explanation of the specific character of the type-II 'canals' involves a very difficult task. Whether they represent an artifact, as suggested by Bergen ('04), or are actually the negative picture of some preformed substance in the living cell is not clear. It must be emphasized that the type-II canals are not consistently found in all spinal-ganglion cells.

My own observations do not permit me to offer a precise explanation as to the origin of these clear spaces, but they do seem to justify the conclusions that they are distinct from the negative image of the apparatus of Golgi, the canalicular apparatus of Cowdry, and the Saftkanälchen of Holmgren. They are probably identical with the clear canals described by Penfield ('21).

VACUOME

The vitally stained neutral-red vacuome seems to have been first described in the vertebrate spinal-ganglion cells by

Covell and Scott ('28). Parat ('28) immediately accepted the condition described for the spinal-ganglion cells by Covell and Scott and finds a powerful confirmation in their work for his vacuome theory, namely, that the apparatus of Golgi is formed by the artificial fusion of neutral-red-stainable granules and vacuoles due to action of the metallic impregnation methods. The classical Golgi apparatus is accordingly considered by these investigators as an artifact; the reality, they claim, is the vacuome.

The vacuome in the spinal-ganglion cells can be caused to appear by the injection intraperitoneally of an aqueous solution of neutral red. The first indication of the appearance of the vacuome is the presence of small red granules. These granules increase in size and number, depending upon the concentration of the neutral red injected and the length of time it is permitted to act before the spinal-ganglion cells are examined. However, it must be pointed out that not all the spinal-ganglion cells of the rat react to the neutral-red injections with the same degree of rapidity and susceptibility.

Neutral-red bodies appear typically of a granular nature and often possess irregular or angular contours (figs. 9, 10, 11). They vary considerably in size, and their distribution appears, as far as I can determine in most cases at least, fairly even throughout the cell. In a few cases I have noted an apparent alignment of the neutral-red granules similar to that described by Covell and Scott (fig. 11). However, I do not interpret this to mean that the condition is in any way preliminary to a coalescence of the neutral-red granules, because in cells which show the apparent alignment of the neutral-red granules one can oftentimes faintly see areas in the cell that appear completely void of the neutral-red bodies. These clear areas are probably the Golgi apparatus.

If an excised vitally stained spinal-ganglion cell is allowed to stand in a sealed preparation for one hour or longer, there frequently appear around the neutral-red granules pink-staining vacuoles. The longer the preparation is permitted to stand, the larger and more numerous the vacuoles appear,

until cytolysis is apparent. These findings, which are in accordance with those of Covell and Scott, would seem to indicate that the vacuoles are perhaps formed in some such manner as by the adsorption of water around the neutral-red granules.

In permanent preparations of the vacuome by injection of neutral red, followed by treatment with modified Mann-Kopsch technique, the granules appear similar in most respects to those of the living cell. Following this technique, they are usually a dark brown color or a deep black. Their contour is spherical or angular and the bodies give the appearance of a dense granular substance (figs. 7 and 8). If the preparations are successful, a Golgi apparatus makes its appearance among the neutral-red osmiophilic bodies (figs. 7 and 8). Its form and position are comparable to those in the uninjected specimens, with perhaps a greater tendency toward forming an incomplete network. However, the fragmentation of the Golgi apparatus after the injection of neutral red into the living animal seems not so marked as that recently reported by Ludford ('30) for the acinar cells of the pancreas.

MITOCHONDRIA

The mitochondria in the spinal-ganglion cells have been described by numerous investigators (Cowdry, '12, '14, '18). However, their presence in the adult nerve cells as described by Cowdry has been questioned by Meves ('10) and Hoven ('10).

My observations of the mitochondria in the spinal-ganglion cells of the rat are mainly in corroboration of those of Cowdry ('14) and others. The mitochondria appear as granules, rods, and filaments and are apparently of a fairly even and constant distribution throughout the cyton (figs. 12 and 13). In preparations of mitochondria by Regaud's technique I have found bodies which appear similar to the 'lipoid globules' of Cowdry (fig. 13). These bodies stain in many respects like the mitochondria. They are spherical in out-

line and slightly larger than the granular mitochondria. The distribution of the 'lipoid globules' is not even, and they often appear in localized areas in the cell. They are rarely impregnated following the Mann-Kopsch technique, which would seem to indicate that they are perhaps a structure closely related to that of mitochondria.

Mitochondria are distinct from the bodies of Nissl, as indicated by the simultaneous demonstration of both of these structures in the same cell. They are also distinct from the canalicular apparatus of Cowdry, at least in the spinal-ganglion cells, which offer exemplary material for the differentiation of the mitochondria and the apparatus of Golgi (compare figs. 1 and 2 with 12 and 13). No one who has had the pleasure of judiciously studying preparations of the mitochondria and Golgi apparatus in the spinal-ganglion cells of the rat can fail to be struck by the complete absence of any form of transition between mitochondria and Golgi apparatus, as erroneously described by Parat and his followers.

THE NISSL BODIES (CHROMATIC SUBSTANCE)

The Nissl bodies in the spinal-ganglion cells of the rat do not differ from those described in many text-books of histology for the spinal-ganglion cells of other mammals (fig. 15). They appear as angular, irregularly shaped bodies which stain deeply with basic dyes. As is well known, their size, number, and position are subject to considerable variation in different cells of the same category.

The Nissl bodies are usually feebly preserved in preparations following Regaud's technique for mitochondria (fig. 13). However, as previously pointed out, they are sufficiently prominent in certain cells to easily dismiss the claim that the mitochondria and Nissl bodies are one and the same structure. In some preparations of the spinal-ganglion cells, following the modified Mann-Kopsch technique, the Nissl bodies and the Golgi apparatus are distinctly shown in the same cell (fig. 3). It is interesting to note, however, that in most cases where the Nissl bodies are clearly shown the Golgi apparatus is

frequently present as an incomplete network. This would seem to be in harmony with the interpretation that perhaps an intracellular stress and strain is set up following the presumed clumping of Nissl substance, that could account for the breaking up the Golgi apparatus.

The Nissl bodies have not been observed in the living cell, and it seems possible that they may be products of an artificial coagulation of a substance which in life is dispersed in the cytoplasm as ultramicroscopic granules (the so-called Nissl substance).

To test the theory that the Nissl substance (chromatic substance) is histochemically identical with chromatin material in the nucleus, I applied the Feulgen reaction, said to be a test for chromatin material (thymus-nucleic acid). The reaction was wholly negative as regards chromatic substance in the cytoplasm and very feeble in respect to the chromatin of the nerve cell nucleus as compared with the chromatin in the nucleus of the capsular cell (fig. 14). Hence, we can conclude that the chromatin material in the nuclei of the spinal-ganglion cells is relatively sparse as compared to that of nuclei of similar size in other tissues.

The important point to be derived from this study of the Nissl bodies is, namely, that they are distinct from the mitochondria, Golgi apparatus, and vacuome. Furthermore, if the Feulgen reaction is a test for chromatin material (thymus-nucleic acid), it is very doubtful that the Nissl bodies are derived from the chromatin of the nucleus.

DISCUSSION

It has been recently urged by Parat and Painlevé ('24, '25), Parat ('28, '29), Zweibaum and Elkner ('30), Ma ('30), and others that the so-called Golgi apparatus (internal reticular apparatus, Binnennetz, Netzapparat, etc.), long ago made known by Golgi, Kopsch, von Bergen, Weigl, Cajal, and others and later studied by many cytologists, has no objective existence in the living cell—that it is indeed an artifact, pure and simple, produced by the methods used for its demonstra-

tion. This theory has found strong support in the work of Covell and Scott ('28) on the ventral-horn and spinal-ganglion cells of the mouse. They declare that their "findings offer, we believe, strong support to Parat's theory that the Golgi apparatus does not exist, as such, in the living cell, but results from the linear arrangement and union of granules and vacuoles, which in the living state have an affinity for neutral red, and from the blackening of the resulting network with silver and osmium." Covell and Scott ('28) were able to study the nerve cells directly under the microscope and particularly to record the appearance in the process of impregnation of the neutral-red-stained vacuome by osmic acid over long periods of time. Apparently they were never able to see the fusion of the vacuoles and granules to form a network after they had been fixed, although a 'curious' alignment was sometimes observed.

In order to accept the view of Covell and Scott and of Ma it is necessary to grant that the vacuoles and granules fuse after they are fixed for long periods of time by 2 per cent osmic acid. Yet it is difficult for the writer to conceive of a migration of granules and vacuoles and their subsequent fusion to form a typical network in a cell that has been completely fixed and coagulated, as is undoubtedly the case after prolonged exposure to 2 per cent osmic acid. Nevertheless, it is upon this very point that the vacuome theory is based and it is this important supposition that Covell and Scott, Parat, and others have failed to adequately substantiate. Furthermore, it has been shown by Gatenby ('31), Hirschler ('28), and many others that the Golgi apparatus and the vacuome are distinct and may occupy two quite separate positions in the cell. Then, too, it has been demonstrated by the writer in the acinar and islet cells of the pancreas that the neutral-red granules do not fuse to form the apparatus of Golgi, as indicated by the simultaneous demonstration of the vacuome and the Golgi apparatus in the same cell. These findings have been recently confirmed by Ludford ('30) and Hirsch ('31) in the acinar cells of the pancreas. Dawson ('31)

has likewise reported that in cartilage cells the neutral-red bodies are distinct from the apparatus of Golgi. Moreover, Cowdry ('12) has demonstrated in the spinal-ganglion cells that—

The canalicular system is, nevertheless, not an artifact produced by the fixing agent because I have frequently been able to demonstrate it by staining intra-vitam by pyronin. . . . Cells may sometimes be seen in which the canals stand out as clear spaces in a very fine granular red-stained cytoplasm.

In my own experiments on the spinal-ganglion cells I have never been successful in demonstrating the Golgi apparatus with neutral red. In addition, it is interesting to note that Parat himself ('28, '29) seems to find a more attractive hypothesis in his 'active' chondriosome theory.

Covell and Scott also found that if they subjected to osmic-acid vapor for twenty minutes a spinal-ganglion cell which (I presume) had been previously stained vitally with neutral red, the vacuoles were found to be fused in some regions. This they interpreted to be an intermediate stage in the formation of the apparatus of Golgi. As a result of my own experiences and the findings of others, I am led to believe that what Covell and Scott described in their figure 16 is not the classical apparatus of Golgi. The evidence to support my conviction is that the Golgi apparatus is seldom demonstrated upon such short exposure to osmic acid. Secondly, similar figures to those of Covell and Scott can be easily duplicated by permitting vitally stained ganglion cells to stand exposed to a foreign environment for considerable time, under which conditions the granules and vacuoles may, in some cases, appear to fuse. Then if the tissue is exposed to 2 per cent osmic acid for a short time, figures such as Covell and Scott's no. 16 can be found. Accordingly, such preparations are considered artifacts produced while the cell is in a foreign environment and subsequently revealed by the osmic-acid technique.

The alignment of the neutral-red granules and vacuoles as found by Covell and Scott is interesting in the light of the

recent evidence presented by Ludford ('30), Krjukowa ('29), and Alexenko ('30) that neutral-red bodies may be secreted by the Golgi apparatus. If this interpretation is correct, it offers a plausible explanation for the alignment of the neutral-red bodies in a topographical distribution corresponding to that of the apparatus of Golgi. However, from my own observations I am not convinced that the neutral-red bodies are secreted by the Golgi apparatus. But in any case it is reasonable to assume that the presence of the Golgi apparatus in the cell mechanically affects the arrangement and distribution if the neutral-red bodies to some degree.

The suggestion of Gatenby ('29) that the findings of Covell and Scott might possibly be explained by the assumption that the Golgi apparatus exists as a thin pellicle on the periphery of the neutral-red-stained canal (vacuome) seems hardly applicable here, since it has been shown that the existence of a neutral-red-stained canalicular network in 'normal' spinal-ganglion cells seems highly improbable. It is the writer's opinion that wherever the fusion of the neutral-red vacuoles and granules simulates the Golgi apparatus in the spinal-ganglion cell it is an artifact in the sense that a vitally neutral-red-stained network does not exist, as such, in the 'normal' living cell.

Recently, Owens and Bensley ('29) have used the spinal-ganglion cells to demonstrate the nature of the Golgi apparatus. They have applied 2 per cent osmic acid to the spinal-ganglion cells after various methods of fixation and have found that they can vary at will the election of the reduced osmium on different structures of the cell, depending on the fixative used. They conclude, therefore, that—

. . . . in the light of these reflections it is obvious that the 'magnificent apparatus of Golgi' as displayed in the osmic and silver impregnations is but a mass of precipitated osmium or silver on surfaces and in spaces preexisting in the cell. The reality is a system of spaces with their enveloping membranes revealed by the techniques of Holmgren, Bensley, Parat and Painlevé and Guilliermond,—in other words the vacuome.

It is true that Professor Bensley's observations are indeed important in that they confirm and emphasize the delicacy of the osmic-acid reaction. However, I have obtained preparations of the spinal-ganglion cells following the Mann-Kopsch technique alone in which certain of the cells possessed what might be termed 'magnificent apparatus of Golgi,' while the neighboring cells displayed many of the variations depicted by Owens and Bensley. What does this mean? The writer interprets such preparations as indicating that the osmic-acid reaction for the demonstration of the Golgi apparatus is probably a delicate one and that the individual spinal-ganglion cells vary greatly in their physiological states—a circumstance that may affect to a certain degree the fixation of the Golgi apparatus. In any case, whatever the interpretation may be, the facts remain that such a condition exists. This is not only true of the Golgi apparatus, but is likewise true to a lesser degree of the mitochondria and vacuome. I have observed in some cases certain spinal-ganglion cells which displayed the mitochondria in apparently every degree of excellency, while adjacent cells presented only negative results as regards the mitochondria.

Bensley, in the same paper, justly reiterates his claim to the discovery of the vacuome hypothesis in 1910 in plant cells and expresses gratitude that it has been so extended by the excellent researches of Guilliermond. He likewise assumes that the vacuome of animal and plant cells is homologous and lauds Parat on his recent work on the vacuome in animal cells. However, if one examines the papers of Parat and Painlevé ('24, '25) and Bensley ('10, '11) he will immediately be struck by the conclusion that the canalicular apparatus of Bensley in animal cells and the vacuome of Parat are two quite distinct structures, and are, therefore, not comparable. Parat recognizes the trophospongium of Holmgren (the negative of which Bensley interprets as his canalicular apparatus) as comparable to the apparatus of Golgi, both of which he declares are artifacts. Furthermore, Parat has consistently urged the view that the vacuome is specifically stained by neu-

tral red, while Bensley ('11), on the other hand, states that the "... canals of Holmgren ... appear as clear spaces among the deeply stained [neutral red] granules." This observation of Bensley's has been confirmed recently by O'Leary ('30) and by Beams ('30). Moreover, in the present experiment I have demonstrated that the bodies displayed by the neutral-red technique of Parat are wholly distinct from the Golgi apparatus and the canalicular apparatus of Bensley and Cowdry. Then, too, Bensley holds that the vacuome in the living cell is in the form of anastomosing canals, while Parat, on the other hand, contends that the vacuome is made up of discrete vacuoles and granules that are specifically stained with neutral red. Therefore, from the results of this study and a review of the papers of Bensley and Parat, it is apparent that what Bensley is calling vacuome in animal cells (canalicular apparatus) is refractive to neutral-red staining and is comparable to the negative image of the apparatus of Golgi. Contrarily, Parat holds that the vacuome is a collection of granules and vacuoles in the cell that are specifically stained by neutral red. It would seem, then, that Bensley is mistaken in comparing his vacuome (canalicular apparatus) with Parat's neutral-red-staining vacuome in animal cells. It is probably true that the vacuolar system described in plant cells by Bensley ('10) is the vacuome of Guilliermond and others. But it still remains to be proved that the vacuomes of animal and plant cells are homologous structures.

Attempts to prove the Golgi apparatus an artifact have been made by Legendre ('05, '10), who states that the Golgi apparatus is merely a deposition of reduced osmium on the Nissl bodies. In other cases he declares that the Golgi apparatus is wholly absent or is the result of pathological changes in the cell. These findings of Legendre seem never to have been seriously considered and are of interest only from a historical point of view.

Holmgren, in a long series of papers, dating from 1899 to 1915, has described and confirmed in a great variety of cells

(including spinal-ganglion cells) the presence of a system of intracellular canals, 'Saftkanälchen' or 'Kanälchen,' which he holds to be a liquefaction of a portion of the protoplasmic network (trophospongium), which is identical with the apparatus of Golgi. As Maximow ('30) has clearly stated—

According to Holmgren, some of the 'highest' types of cells of the organism and in particular the nervous cells, which require special arrangement for abundant nourishment, enter into a peculiar symbiotic relationship with other 'lower' elements, which nourish the 'highest' elements and which, therefore, may be called the trophocytes. Special elements lying on the surface of the nerve cell give off long processes which enter the cell, branch and anastomose here in the form of a 'nourishing' net or 'trophospongium' and penetrate the protoplasm between the tigroid globules and neurofibrils.

Holmgren suggests ('15) that the trophocytes absorb substance from the blood in the immediate vicinity of the spinal-ganglion cells, and store them in their cell body in the form of a material that may be darkened by osmic acid. From the trophocyte the substance passes directly into the process of the trophospongium of the spinal-ganglion cell. Holmgren contends that the Saftkanälchen is the negative image of the Golgi apparatus and that it is formed by the coalescence of droplets of liquid in the substance of the trophospongium, which thus forms a canal within the thread until it is completely replaced by a trophospongium canal or 'Saftkanälchen.' He states further that the osmic and silver methods do not always completely impregnate the trophospongium, so that the extensions of the trophospongium (Golgi apparatus) to the surface of the cell were not observed by Golgi and other workers who used only the silver and osmic methods.

If one compares the figures of Holmgren ('14) on the trophospongium of the spinal-ganglion cells with those presented in this paper for the Golgi apparatus in spinal-ganglion cells, it is obvious that the two are identical.³ However, Holmgren ('14, '15) insists that the trophospongium reaches the surface

³ There appear unquestionably in certain invertebrate nerve cells structures which penetrate the cells from the periphery. A discussion of these structures will appear elsewhere.

of the cell and that its function is chiefly nutritive. I have been unable to confirm this view of Holmgren's and my observations support the view of Golgi ('98, '99), Kopsch ('02), von Bergen ('04), Cowdry ('12), Penfield ('21), and others that the Golgi apparatus is entirely intracellular. A more serious problem presents itself as to whether or not the Saftkanälchen of Holmgren, clear canals of von Bergen, and canalicular apparatus of Bensley and Cowdry are all one and the same structure. Likewise, are these structures in reality the negative image of the Golgi apparatus? On the basis of the evidence presented in this paper I am convinced that the canalicular apparatus represents the cytoplasmic mold of which the Golgi apparatus forms the cast and, further, that the Saftkanälchen of Holmgren, clear canals of von Bergen, and canalicular apparatus of Cowdry all represent one and the same structure in the spinal-ganglion cells. However, no extensions of the canalicular apparatus to the surface of the cell as claimed by Holmgren were observed. This interpretation is in harmony with that of Kopsch, Golgi, von Bergen, Cajal, Cowdry, Duesberg, Gatenby, Bowen, Ludford, and others. Then, too, it is in accordance with what is known to be true for the mitochondria as shown by Bensley ('11) and Saguchi ('20) and is in agreement with the view that the Golgi apparatus is lipoidal in nature. Cowdry ('24) has demonstrated in the same cell of the acinus of the pancreas that the Golgi apparatus can be impregnated, mounted, drawn under the camera lucida, bleached, and stained with hematoxylin; following which, in place of the Golgi apparatus, there appears a canalicular apparatus, which it seems to the writer is beyond doubt the negative image of the apparatus of Golgi. Holmgren ('14) has made similar experiments on the spinal-ganglion cells.

In a recent study Penfield claims to have shown in the spinal-ganglion cells, by use of Cajal's silver-nitrate impregnation method and subsequent staining with Heidenhain's hematoxylin, the simultaneous occurrence, in the same cell, of the Golgi apparatus and 'trophospongial canals' of

Holmgren.' Hence he concludes that they are distinct and separate structures. Moreover, Penfield claims that after sectioning the nerve trunk and examining the corresponding spinal-ganglion cells, the Golgi apparatus migrates from its normal position to a region near the periphery of the cell (retispersion), where it sometimes undergoes dissolution (retisolution). He found, however, that this was not the case with the 'trophospongial canals of Holmgren,' as they seemed to retain their regular position regardless of nerve injury. Penfield accordingly concludes that the "Holmgren canals are not simply the negative of the Golgi apparatus."

About the same time Da Fano ('21) reported his results on an experimental study of the Golgi apparatus in the nerve cells of the spinal cord of the rat. He found after exposure to low temperature that—

... in the more affected specimens the apparatus gradually loses its characteristic structure and becomes transformed into irregular and variously arranged masses and pieces. These alternations are accompanied by an appearance of canaliculi and spaces similar to those generally spoken of as Holmgren's 'trophospongium.'

The writer has in the preceding observations presented evidence which he thinks is comparable to that of the clear canals of Penfield and the condition presented by Da Fano. These 'canals' appear simultaneously in preparations which show distinctly the Golgi apparatus and vacuome. They are not the negative simulacrum of the Golgi apparatus. They are not, judging from the figures of Holmgren and Holmgren's own interpretation, the same as his Saftkanälchen, and they are not, as Cowdry maintains, the same as the canalicular apparatus. Furthermore, my own observations show that there may be demonstrated in the spinal-ganglion cells two types of clear 'canals,' viz., the canalicular apparatus or Saftkanälchen and the clear canals of Penfield. The former represents the negative image of the apparatus of Golgi, while the latter is distinct from the canalicular apparatus. If this interpretation is correct, it will easily account for the wide discrepancies in the views presented by Penfield and Da Fano.

The trophospongial canals of Penfield and the writer's type-II canals recall the figures of von Bergen type-II canals which he held were artifacts and could never be positively stained. In a careful study of the figures of von Bergen type-II canals, 'trophospongial canals' of Penfield, and the type-II canals described in this paper the writer is convinced that they probably represent the same structure. From the evidence presented by Penfield and Da Fano it would seem evident that the appearance of these canals is accentuated under the experimental condition of nerve section and exposure to low temperatures, while in my own preparations 'canals' similar to those described by Penfield and Da Fano were observed in spinal-ganglion cells from animals which, from all appearances, were perfectly normal. It is true, however, that the percentages of nerve cells which showed the type-II canals were greater in those ganglia of animals that had been injected with neutral red. Da Fano has accepted the suggestion of Nageotte that the clear canals in his preparations may be the exaggeration of a normal condition, brought about by a certain degree of edema, possibly due to a vasomotor change resulting from exposure to cold. It is obviously very difficult to determine whether or not the clear trophospongial canals of Penfield and Da Fano and type-II canals are artifacts, as suggested by Bergen, or are actually preformed structures in the cell. The final answer to this question, for the present, at least, must await further investigation. But in any case the important knowledge to be gained from this study is that the trophospongial canals of Penfield and Da Fano are very probably not the same as the Saftkanälchen of Holmgren, type-I canals of von Bergen, and the canalicular apparatus of Cowdry, but represent an entirely different structure which may coexist in the cell with the Golgi apparatus.

The mitochondria in nerve cells seem to have been figured long ago by Altmann, who depicted structures among which are undoubtedly what we now term mitochondria. Altmann called them bioplasts (living units), and it was not until the

studies of Benda ('87) on the histogenesis of spermatozoa that we were given the term mitochondria (thread-like granules). To Cowdry must go much credit for carefully demonstrating the independence of mitochondria and Nissl bodies in the spinal-ganglion cells of a wide variety of animals. He has insisted that the mitochondria are of constant occurrence in the adult spinal-ganglion cells of vertebrates in the form of granules, rods, and filaments—a condition which has been questioned by Meves ('10) and Hoven ('10). Moreover, as Duesberg ('20) points out, Monti ('15) was unable to distinguish between Golgi apparatus and chondriosomes in neurons. However, the advent of the use of Janus green B as a vital stain for mitochondria and the studies by Cowdry and others on the mitochondria in the spinal-ganglion cells of a wide variety of vertebrates have left little to be desired as regards their occurrence and morphology. The observations presented in this paper are merely confirmatory of the findings of Cowdry and they substantiate the view that the mitochondria show no obvious relationship to the internal reticular apparatus of Golgi and the Nissl bodies.

Very recently an interesting evolution of the mitochondrial theory has been developed by Parat. By using polarized cells and the potassium bichromate-hematoxylin technique, he finds an area rich in lipoid which he terms the 'zone of Golgi' and contends that it is composed in part of special or 'active' chondriosomes that are directly derived from the mitochondria by the accumulation of lipoids. Parat asserts that the 'active' chondriosomes have been mistaken by many investigators for the Golgi apparatus. Inasmuch as Parat has never described the 'active' chondriosomes in the spinal-ganglion cells, so far as the author is aware, it is difficult to postulate their specific character. Nevertheless, in any case, we can say with considerable degree of assurance that the vital method of Janus green B and the permanent methods of Regaud and Bensley stain only short rods and granules in the cytoplasm of the spinal-ganglion cells. Furthermore, it is of especial interest to note, as Cowdry ('12) points out, that von Bergen

used the potassium bichromate-hematoxylin method and found that it demonstrated the Golgi apparatus in every detail comparable to that of the method of Kopsch. Then, too, it has been shown repeatedly, in many polarized cells at least, that there are fewer mitochondria in the region of the Golgi apparatus than in other parts of the cell. Hence, from the evidence at hand it is apparent that the 'active' chondriosomes of Parat are merely what others have called Golgi apparatus for over thirty years.

From the preceding observations and considerations on the nature of the Golgi apparatus and mitochondria I question the wisdom of using the term 'active' chondriosome to designate the apparatus of Golgi. The term seems to the writer ill chosen and unnecessary and better consigned to the long list of less appropriate and unaccustomed synonyms of the Golgi apparatus given by Cowdry ('21). However useful the term 'zone of Golgi' as pointed out by Gatenby ('30) and Dawson ('31) may be in certain cases, it is inapplicable to the condition that exists in the spinal-ganglion cells.

None of the supposed elements of the cytoplasm of the nerve cells have received wider attention than the so-called chromatic substance, Nissl bodies, or tigroid bodies first described by Flemming in 1882, according to Cowdry ('12), and later extensively studied by Nissl and many others. The present question, which concerns the morphology of the Nissl substance, is whether or not it exists in the living state in the form of isolated flakes and granules or is in reality present in the living cell in a dispersed phase which coagulates into bodies upon fixation. Likewise, is the Nissl substance of nuclear origin, and is it a specific substance in nerve cells? The majority of evidence (Cowdry, '28) seems to favor the view that the Nissl substance exists in the living cell in a dispersed state and is coagulated into large irregular-shaped bodies following certain fixations. On the other hand, recent evidence by Wiemann ('25) seems to have revived the view that the Nissl bodies are preformed isolated structures as indicated by the use of ultraviolet photography. My own

observations, based on the study of the living cell and upon the variations in the appearance of the Nissl substance following exposure to different fixing solutions, are in accordance with the view that the Nissl bodies probably do not exist as such in the ganglion cell. That there is a substance in nerve cells which forms the Nissl bodies seems highly probable, as indicated by the varying amount present at different physiological states of the cell.

As regards the origin of the Nissl substance from the chromatin of the nucleus, my observations are in accordance with the view that the Nissl bodies are of cytoplasmic origin. I have applied the Feulgen nucleal reaction to the spinal-ganglion cells with totally negative results as regards the Nissl bodies. This is in agreement with the findings of Wermel ('27). Thus, if Feulgen's reaction is specific for chromatin material (thymus-nucleic acid), then we can conclude that the Nissl bodies are probably not chromatin material. Furthermore, it is of interest to note that the spinal-ganglion cells, like the oocytes (Ludford, '28), show apparently less chromatin material in the nucleus, as indicated by the Feulgen reaction, than do many other varieties of cells. Also, the spinal-ganglion cells give a wide range of variation in nucleoplasmic ratio according to the formula of Hertwig. Inasmuch as it seems highly improbable that the Nissl bodies are histochemically the same as chromatin, the suggestion of Heidenhain (Kuntz, '28) that the variation in karyoplasmic ratio in nerve cells could be accounted for by assuming the Nissl bodies are of chromatin origin seems doubtful.

No study of the centrosomes, neurofibrils, pigment granules, or the neurosomes of Held has been included in this investigation. The preponderance of evidence at present seems to favor the view that the neurofibrils are quite distinct from any of the above-named cytoplasmic components.

It has been repeatedly pointed out by numerous investigators that great care and precaution should be exercised in the interpretation of cellular components following the fixing

and staining of preparations. As a matter of fact, in some quarters considerable doubt has been reflected on all structures that cannot be demonstrated in the living cell. It is possibly true that the formation of the Nissl bodies exemplifies a case of the actual appearance of an artifact. However, it seems unquestionable that there is a substance (Nissl substance?) which forms the basis of the artifact in nerve cells. It must be remembered, however, that if this interpretation is correct it does not necessarily imply that other structures in the cell should react to the coagulation phenomena to the same marked degree. In this connection it should be emphasized that the majority of cellular structures which can be observed in the living cell have been shown over and over again to be in every way comparable to the main body of evidence gained through the study of fixed and stained preparations. Nearly all the important information regarding chromosomes, mitochondria, and achromatic figure has been repeatedly confirmed by the more recent vital methods. Accordingly, it seems to the writer improper to consider structures in the cell demonstrated by fixed and stained preparations as artifacts in the broad sense of the word, that is to say, that there is not a different physical chemical condition in the protoplasm of the living cell which upon coagulation presents a refractive index different from that of the surrounding substance. On the other hand, it is pertinent to point out that the use of the vital methods offers, we believe, even greater dangers in the formation of artifacts than that of the classical methods. From the recent works of Nassonov ('26), Chlopin ('27), Krjukowa ('29), Dawson ('29), Beams ('30), Ludford ('30), Alexenko ('30), and others, it is becoming increasingly apparent that many of the bodies colored by vital dyes that have been held to be preexisting are simply new formations in the cell. Furthermore, wherever fusion of the neutral-red bodies has been observed to take place, it probably constitutes, in many cases at least, an artifact.

CONCLUSIONS

1. The Golgi apparatus may appear in the spinal-ganglion cells in the form of a typical paranuclear network, as an incomplete reticulum, or as isolated filaments of osmiophilic substance. The Golgi apparatus is entirely cytoplasmic and appears as a constant cellular component. It is interpreted as identical with the trophospongium of Holmgren ('14), but Holmgren's notion that the Golgi apparatus (trophospongium) penetrates the surface of the cell is thought to be in error.

2. The Golgi apparatus is not stained in the spinal-ganglion cells by neutral red.

3. The canalicular apparatus of Cowdry, Saftkanälchen of Holmgren, and the type-I canals of von Bergen are thought to represent one and the same structure in the spinal-ganglion cells of the rat, and are interpreted as the negative image of the apparatus of Golgi. Like the Golgi apparatus, these structures have not been observed to connect with the surface of the cell.

4. The type-II canals of von Bergen and clear canals of Penfield are interpreted as distinct from the canalicular apparatus, Saftkanälchen, or negative image of the apparatus of Golgi.

5. The vacuome appears in the spinal-ganglion cells in the form of isolated granules of variable size and number, depending to some degree on the amount of neutral red injected, its concentration, and the time it is permitted to act. These granules do not fuse to form the apparatus of Golgi as urged by Parat and by Covell and Scott, as indicated by the fact that the Golgi apparatus and the neutral-red bodies can be demonstrated to coexist in the same cell.

6. Mitochondria of the spinal-ganglion cells appear as granules, filaments, and rods. They are evenly distributed throughout the cell and show no evidence whatsoever of intermediate stages in the formation of the apparatus of Golgi ('active' chondriosomes of Parat), as Parat's most recent theory would seem to necessitate.

7. The Nissl bodies are found as large irregularly shaped masses, of considerable variation in size and number, in different cells of the same ganglion. They do not give a positive reaction with Feulgen's method, thereby indicating that they are histochemically different from the chromatin material of the nucleus. They are distinct from the mitochondria, Golgi apparatus, and vacuome.

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PLATE 1

EXPLANATION OF FIGURES

1 Spinal-ganglion cell of the rat, showing the typical apparatus of Golgi. A portion of the capsule is shown on the periphery of the cell. (Modified Mann-Kopsch (Weigl) technique.)

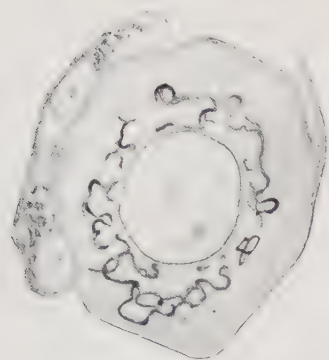
2 A cyton of the spinal ganglion viewed in toto. The strands of the Golgi apparatus appear continuous as far as they can be followed into the depths of the cell. (Modified Mann-Kopsch (Weigl) technique.)

3 Cell of the spinal ganglion which shows clearly the Nissl bodies, and the Golgi apparatus as discrete filaments. (Modified Mann-Kopsch (Weigl) technique.)

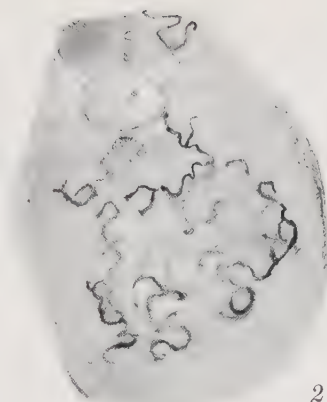
4 A cell in which the complete dissolution of the Golgi apparatus into granules and vacuoles is apparent. This cell was found in a spinal ganglion in close proximity to cells which display the typical apparatus of Golgi. (Modified Mann-Kopsch (Weigl) technique.)

5 Portion of a section of the spinal ganglion prepared according to Regaud's formol-potassium bichromate-hematoxylin technique. Clear spaces are present (canalicular apparatus of Cowdry, Saftkanälchen of Holmgren, and type-I canals of von Bergen) which resemble closely the form, distribution, and intracellular position characteristic of the Golgi apparatus. Mitochondria are also shown in these cells as short rods and filaments.

6 Cyton in which the Golgi apparatus and clear canals of Penfield ('21) are demonstrated in the same cell. The clear canals of Penfield are interpreted as quite distinct from the canalicular apparatus of Cowdry, Saftkanälchen of Holmgren, and the type-I canals of von Bergen.



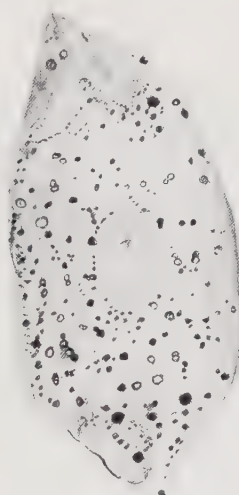
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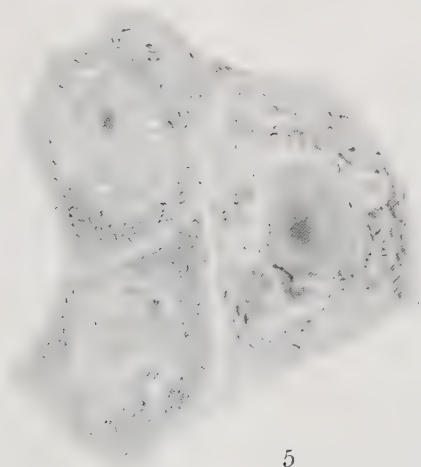
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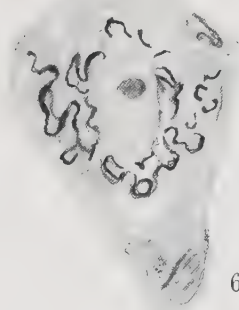
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PLATE 2

EXPLANATION OF FIGURES

7 Two spinal-ganglion cells which show distinctly the vacuome (neutral-red osmiophilic bodies), Golgi apparatus, and clear canals of Penfield ('21). These structures are undoubtedly distinct. (Intravital-stained with neutral red, followed by modified Mann-Kopsch (Weigl) technique.)

8 Spinal-ganglion cell in which the Golgi apparatus and vacuome are clearly and distinctly shown in the same cell. (Intravital-stained with neutral red, followed by modified Mann-Kopsch (Weigl) technique.)

9 to 11 Drawings from preparations of living spinal-ganglion cells stained intravitaly with neutral red. The neutral-red bodies (vacuome) are shown in their usual distribution. In figure 11 an apparent alignment of the neutral-red bodies is observed. This condition is not often discernible, however. These bodies are identical with the neutral-red osmiophilic bodies depicted in figures 7 and 8.

12 and 13 Cells which show typically the form and distribution of the mitochondria in the spinal ganglion. In figures 13 large spherical granules are noted which are interpreted as the 'lipoid globules' of Cowdry ('14). (Regaud's technique.)

14 A cyton treated according to the 'Nuclealreaktion' of Feulgen. The cytoplasm appears homogeneous and there is no indication whatever of a positive reaction with the Nissl bodies (chromatic substance). (Feulgen's method, as given by Ludford, '28.)

15 A cell of the spinal ganglion which shows the Nissl bodies in their typical appearance. (Delaney, '27, method.)



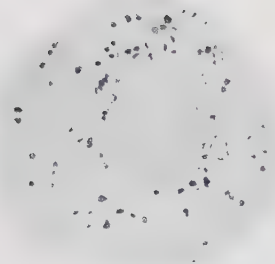
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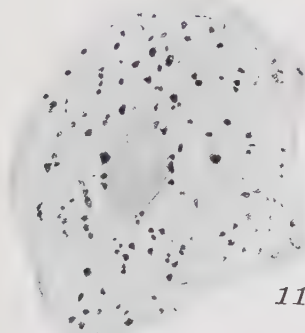
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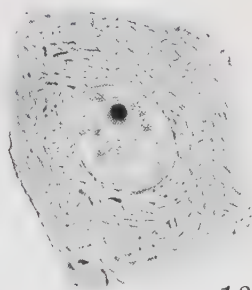
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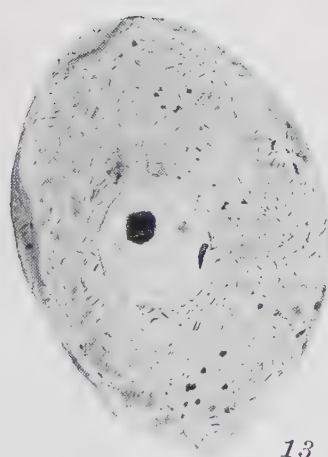
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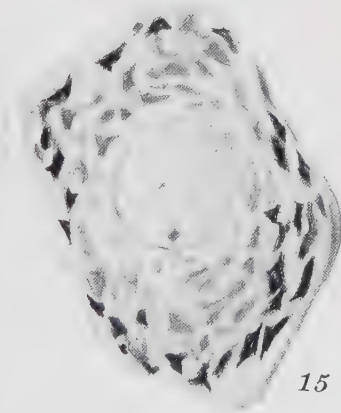
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STUDIES ON AMPHIBIAN ENDOCRINES

II. THE PITUITARY GLAND OF *NECTURUS MACULOSUS*

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ONE TEXT FIGURE AND TWO PLATES (THIRTEEN FIGURES)

Literature describing the detailed morphology, histology, or cytology of the hypophysis of Amphibia is surprisingly sparse. Müller ('71), Kupffer ('94), Valenti ('95), Kingsley and Thyng ('04), Haller ('09), Tilney ('11), Stendell ('14), Atwell ('18, '21), Sumi ('24, '26), Pokorny ('26), and De Beer ('26) are credited with giving us our present knowledge of the morphogenesis and morphology of the amphibian pituitary. Of these workers, only four (Tilney, Stendell, Atwell, and De Beer), have attempted any extended histological investigations of this gland in urodeles. Three of them (Tilney, Stendell, and Atwell) have described, in part, the morphology and histology of the pituitary body in *Necturus*. Reference to the cytology of the various elements of the hypophysis of Amphibia apparently has been limited to a description of their staining reaction and general nature of their cytoplasm.

As previously indicated (Charipper, '29), the author has undertaken to ascertain the normal anatomy, histology, and cytology of both the thyroid and the pituitary gland in a permanently neotenic amphibian. In view of the demonstrated importance of the interrelated activity of these glands, special attention had been directed to morphological evidences of physiological activity. The present work is confined to the pituitary gland alone. Results of the investigation of the thyroid gland have been published in a separate paper.

This problem was carried on at the biological laboratories of New York University at the suggestion of Prof. Alden B. Dawson, to whom acknowledgment is made for his kindly interest.

MATERIAL AND METHODS

The fresh material studied was obtained from animals sent to New York from Pennsylvania and the Ithaca region of New York state. The animals ranged in size from 22 to 30 cm. in length. The gross topography of the pituitary was obtained entirely from fresh animals. The whole brain was fixed in Helly's or Schaudinn's fluid after decapitation and appropriate trimming of the brain case to permit ready access of the fixative. This entire operation was done in less than a minute. After fixation and washing, the hardened brain was removed without mutilation. The general position and topography of the pituitary were checked in sixty full-grown, commercially prepared, formalin-fixed specimens.

Material for histological and cytological examination was prepared from seine-caught animals which in no case had been in the laboratory longer than six days. The decapitation method was used and the trimmed cranium containing the entire brain was fixed in Zenker's, Bouin's, Zenker-formic-osmic, Nasonov's modification of Kolatchev's, Champy's, Mann-Kopsch's, or Schaudinn's fixing fluid. For general histological routine haematoxylin-eosin, Heidenhain's iron haematoxylin, and Nocht-Romanowsky's stains were used. For cytological material Nasonov's modification of Kolatchev and Mann-Kopsch methods were found to give the best results for the osmiophilic, formed elements of the cytoplasm. Iron haematoxylin following a Mann-Kopsch fixation gave the best results for granules. The slides were prepared from paraffin-embedded tissue cut 5 to 7 μ thick. Frontal, sagittal, and transverse serial cross-sections were prepared.

THE ANATOMY OF THE PITUITARY GLAND

The following description of the general anatomy and morphological relationships of the pituitary body in the adult

Necturus maculosus is based on observations of this gland in situ, as well as on the examination of serial sections through the transverse, sagittal, and frontal planes of the hypophysis. The results of the present investigation are generally in accord with the observations reported by other authors.

The fully developed pituitary gland in *Necturus* rests upon the flattened base of the brain case, immediately beneath the mesencephalon. The position and relation of the hypophysis in *Necturus*, consistent with observations in other urodele

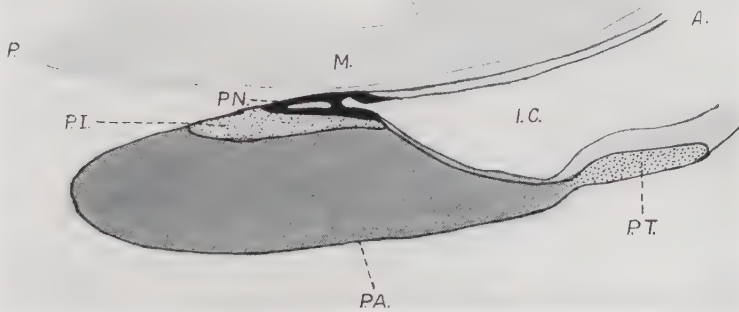


Fig.1 A diagrammatic parasagittal section through the hypophysis of a fully grown *Necturus*, showing the various parts in relation to one another. $\times 36$. A., anterior; I.C., infundibular cavity; M., mesencephalon; P., posterior; P.A., pars anterior; P.I., pars intermedia; P.N., pars nervosa; P.T., pars tuberalis.

Amphibia, are considerably different from those found in higher vertebrates. As already described by Tilney ('11) for *Diemyctylus*, the pituitary body in *Necturus* gives the impression of having been forced backward by the processus infundibuli, so that this latter structure, instead of occupying its customary position behind and somewhat above the pituitary gland, lies in front of it (fig. 1). An examination of the ventral surface of the brain shows the hypophysis to be grossly composed of an ovoid mass connected to the ventral surface of the infundibulum by two slender, elongate

bodies, one on each side of the midline. A longitudinal section through the ovoid body under low magnification shows it to consist of three different parts: a main mass, the pars anterior; a thin plate-like layer of cells lying dorsally, the pars intermedia; and a sacculated portion situated more dorsally and somewhat anteriorly, the pars nervosa (fig. 1). The elongate bodies to each side of the midline make up the pars tuberalis (*P.T.*).

THE HISTOLOGY OF THE PITUITARY GLAND

The histological examination of the pituitary gland of *Necturus* presents a most complex picture. While the cells making up the pars tuberalis, pars intermedia, and the pars nervosa are uniform in appearance, the pars anterior presents for critical examination a varied assortment of cellular elements with varied staining affinities. It is perhaps best to consider first those portions of the gland which are more simple histologically.

The pars tuberalis is merely a pair of elongate masses of basophile cells without any characteristic features. It is non-vascular tissue, with round or oval cells containing an ovoid, vesicular nucleus which practically fills the entire cell. The narrow rim of cytoplasm is homogeneous and stains lightly with an excess of eosin, light blue with Nocht-Romanowsky, and light yellow when exposed to osmic acid.

The pars intermedia seen in transverse section appears as a thin line of compact basophile cells (fig. 2) situated between the pars nervosa and the pars anterior. In frontal serial sections this portion of the gland can be identified as a thin, expanded, flattened layer of cells closely attached to the anterior, ventral wall of the pars nervosa and adherent to the dorsal surface of the pars anterior (figs. 1, 2). The cells of the pars intermedia of the pituitary in *Necturus* are identical, in size, shape, and staining reaction, with the cellular elements of the pars tuberalis. The pars intermedia is practically non-vascular. A few isolated blood cells, indicative of a scanty capillary invasion, can be found scattered between the basophiles, chiefly near the pars anterior.

The pars nervosa or neural lobe of the pituitary is very much sacculated. In transverse sections the cavity of this part of the gland may be cut many times in the same section. Atwell ('21) pictures a transverse section through the hypophysis of *Necturus* in which the cavity of the neural lobe has been cut five times (fig. 17, p. 385). The photomicrograph in the present work (fig. 2) shows this cavity cut three times. The walls of the pars nervosa show little differentiation. They contain a few blood vessels which course in between the mossy neuroglia cells and between the ependymal cells. The nucleated ends of the ependymal cells are located close to the edge of the wall, lining the cavity (fig. 2).

The pars anterior of the hypophysis is the most vascular portion of the gland. In longitudinal section this portion of the gland is seen to consist of cords of cells separated by long capillary channels. The cords are made up of three different kinds of cells. The most common variety are large cuboidal or columnar cells which contain a spherical or oval nucleus embedded in a granular cytoplasm. They show a marked affinity for acid stains. Their distinct boundaries and entire conformation associate them with the acidophilic cells described for higher vertebrates (figs. 4, 6). They stain deeply with eosin, red with Nocht-Romanowsky, and dark brown or black when exposed to osmic acid. These cells usually border on the vascular lumen and are so oriented that the nucleus is farthest away from the blood sinus (figs. 4, 6). Deeper in the cords and not bordering on the blood vessels there occur typical basophile cells similar in appearance to those already described for the pars tuberalis and pars intermedia. These basophile cells also form a narrow zone at the periphery of the pars anterior. This narrow zone exists all along the periphery except anteriorly, where the pars anterior approaches the infundibulum. In this region there occurs a narrow zone of tall columnar eosinophiles arranged as a simple columnar epithelium. A third type of cell is found, sparsely scattered through the pars anterior. These are large cells, each containing a chromophobic vacuole (figs. 10, 12,

13, 15). The cytoplasm of these cells stains very lightly with eosin and a light pink-blue with Nocht-Romanowsky. When exposed to osmic acid, the cytoplasm of these cells takes on a light brown tint. Because of their poor staining reaction and the presence of a large chromophobic vacuole, the term 'chromophobic cell' has been considered adequate and will be used in reference to this cell. This is probably in violation of the proper use of that term as understood by the earlier workers who designated the basophile cells as chromophobic. But, in view of the present tendency (Jordan, '27, p. 577) of recognizing those cells which stain only faintly or with great difficulty with either acid or basic stains, as true chromophobic cells, the use of the term as designated seems to be permissible. There is still another type of cell found in the anterior part of the hypophysis. This cell is similar in staining reaction to the basophiles, but is about four times as large. The cell contains a large nucleus which practically fills it, leaving only a very narrow rim of cytoplasm.

The examination of numerous series of sections failed to disclose any trace of a residual lumen or of intra-epithelial deposits of colloid.

THE CYTOLOGY OF THE PITUITARY GLAND

The normal cytological constituents of the cells of the pituitary gland in *Necturus* consist of large and small granules, 'secretory' spheres, large and small vacuoles, granular mitochondria, osmiophilic rings, and some osmiophilic granular substance. As in the case of the histology of the gland, the pars intermedia and the pars tuberalis, consisting of basophile cells alone, are the simpler portions of the hypophysis to consider. The basophile cells in these portions of the gland, and in the pars anterior as well, show a narrow rim of cytoplasm. A few isolated medium-sized granules are present which blacken with iron haematoxylin following a Zenker-formic-osmic fixation. Many finer granules can be found close to the nucleus. These stain light brown with the Mann-Kopsch method and can be readily bleached with

hydrogen peroxide. These are probably fine granular mitochondria. Several larger granules are also present between these finer granules near the nucleus. These larger granules blacken when exposed to osmic acid following a Mann-Kopsch fixation and show a strong resistance to bleaching with hydrogen peroxide. These are perhaps granules of what is generally called Golgi material. No osmiophilic rings were observed in these cells.

The pars anterior, cytologically, is the most complex portion of the pituitary gland of *Necturus*. It is in this portion of the gland, which must be remembered as containing eosinophilic and 'chromophobic' as well as basophilic cells, that a wide and varied assortment of cytoplasmic constituents occur. It is perhaps best to consider each type of cell and its variations separately.

The basophile cells, which usually occur deeper in the cords of the pars anterior and also in the special peripheral zone already described, are similar in cytological appearance to those described in the pars tuberalis and the pars intermedia.

Each of the 'chromophobic' cells, which occur sparsely scattered through the anterior part of the hypophysis, contains a single chromophobic cytoplasmic vacuole situated to one side of the nucleus (figs. 12, 13, 15). In some cases this chromophobic vacuole distends the cell, leaving only a narrow rim of cytoplasm surrounding the vacuole and the nucleus (fig. 15). Numerous osmiophilic rings and granules can be observed in the cytoplasm of such cells fixed in the Mann-Kopsch fluid and exposed to osmic acid. The fine granules which are light brown appear to be distributed in the cytoplasm at the periphery of the vacuole and seem to have a primary condensation in the cytoplasm immediately between the vacuole and the nucleus and a secondary condensation in the cytoplasm close to the opposite side of the nucleus (fig. 13). The position, number, and size of the osmiophilic rings are variable. In those cells in which the vacuole is larger the osmiophilic rings appear closer to the nucleus and are collected in the cytoplasm on the side of the nucleus adjacent to the vacuole (compare figs. 12, 13, and 15).

The eosinophiles, large cuboidal or columnar cells with distinct boundaries and usually bordering on a vascular lumen, show the greatest cytoplasmic differentiation of any of the cells found in the hypophysis of *Necturus*. Small and large granules, 'secretory' spheres, vacuoles, and osmiophilic rings can be observed as the cytoplasmic constituents of the eosinophiles in a single microscopic field under high dry magnification (fig. 7). Cytologically, four varieties of eosinophiles can be recognized. One variety contains small granules near the nucleus grouped in the cytoplasm toward the vascular lumen (fig. 5). A second type contains large spherical granules almost filling the cytoplasm on the side of the nucleus near the vascular lumen. Occasionally one or two granules occur at the opposite pole (figs. 3 *A*, 7). The third variety of eosinophile has a highly vacuolated cytoplasm (figs. 3 *B*, 7). The fourth type of cell contains many large spherical bodies (fig. 8). The end of this cell nearest the vascular lumen is sometimes observed ruptured, with its spherical bodies mingled with the blood cells. The spherical bodies found in these cells have been designated, for purposes of description, as 'secretory' spheres. In anterior-hypophyseal tissue prepared by the Mann-Kopsch method and exposed to osmic acid, numerous blackened rings and granules can be found in the cytoplasm of the eosinophiles (figs. 9, 14). The same is true for cells examined in tissue prepared according to Nassonov's modification of Kolatchev's method (figs. 10, 11). The rings perhaps represent the substance described as Golgi material (Addison, '16; Reiss, '22; Atwell, '29). The granules may be either modified mitochondria or isolated portions of Golgi substance.

DISCUSSION

The anatomical and general histological observations of the hypophysis in the full-grown *Necturus* presented here are in general accord with the descriptions of that gland to be found in the literature (Tilney, '11; Stendell, '14; Atwell, '22). The posterior position of the pars anterior, the possible rela-

tionship of the sacculated pars nervosa to the saccus vasculosus in the Pisces, and the persistent attachment of the pars tuberalis to the pars anterior in the adult animals are characteristic of the gland in urodele Amphibia and have all been adequately discussed by the workers listed above. The flat disc-like conformation of the pars intermedia has been described for *Proteus anguineus* and *Necturus maculosus* (Stendell, '14) and the absence of any residual lumen also has been discussed by Tilney ('11), Stendell ('14), and De Beer ('26). The general anatomy and histology of the hypophysis in the adult *Necturus* as described in the present work are offered here merely as an added confirmation of the already accepted facts concerning this gland in urodele Amphibia.

An examination of the finer histology and cytology of the pars intermedia and the pars tuberalis yields nothing which may serve as evidence of secretory activity. The pars anterior, however, presents for examination and interpretation a varied assortment of histological and cytological entities which may be considered as evidences of cellular activity. The large chromophobic cells, each with its single vacuole, are perhaps storage cells of some kind. The exact nature of the substance filling these vacuoles is as yet unknown. Specific staining tests may show these vacuoles to be filled with a colloid substance. However, regardless of the nature of the substance contained in the vacuoles of the 'chromophobic' cells, they are similar to the storage cells found in tissue in general. Their infrequency, however, is probably an indication that the part they play in the activity of the entire gland is small.

The osmiophilic substance described in the eosinophiles gives the impregnation reaction of Golgi material and, in part at least, fits the description given for the Golgi apparatus in the cells of the pituitary gland in the albino rat, cat, dog, and calf (Addison, '16, albino rat; Reiss, '22, calf, cat, dog; Atwell, '29, cat). Though no definite relationship can be stated, at the present time, for the position of the osmiophilic

substance and the type of cell in which it is found in the pars anterior of the pituitary in *Necturus*, the variations in amount, size, and distribution of the osmiophilic material may be considered as an indication of cellular activity. This interpretation of the significance of the osmiophilic substance as found in the cells of the pituitary of *Necturus* (assuming that it is similar to what the earlier workers have called the Golgi apparatus) is in accordance with the expressed opinions of Cowdry ('22), Wilson ('25), and Bowen ('24).

The occurrence of the four varieties of eosinophiles in the pars anterior is perhaps the strongest histological evidence of cellular activity. If all four types are genetically related and each variety merely represents a stage in a secretory cycle, then it may be assumed that the secretory antecedents are first laid down as granules (fig. 5), these granules increase in size, liquefy, and occur as small vacuoles (figs. 3, 7), the contents of which find their way into the lumen of the capillaries. Another possibility is for the granules to increase in size and be evacuated into the intercellular spaces or blood capillaries as large secretory spheres (fig. 8) which later liquefy. The plausibility of this latter conjecture, however, depends, first, upon proof that the occurrence of ruptured eosinophiles containing the 'secretory' spheres is not due to mechanical injury of the tissue and, secondly, that this cell is a primary cellular element of the tissue of the pars anterior, and not merely one of the wandering coarse granular eosinophile cells (*Schollenleukozyten*) of the blood stream which are known to secondarily invade body tissues (Maximow, '24; Dawson, '27). The presence of secretory antecedents in the form of granules which lose their staining reaction or liquefy is the more common histological manifestation of secretory activity.

This conception of the mode of secretion of the pituitary in a neotenic amphibian ascribes the dominant rôle to the eosinophile cells. This is in accord with the observations of Kraus ('14), Stewart ('22), and De Beer ('26) in their studies on the pituitary of mammals. In this regard it is interest-

ing to note that Collin ('28) considers the basophile (in man and dog) as the most important secreting element of the hypophysis.

The variations of osmiophilic material described in cells of the pars anterior of the pituitary gland of *Necturus*, together with the presence of cytoplasmic vacuoles and granules, both of which show definite variations in size and distribution, are interpreted as histological evidence of secretory activity. Implants of anterior-pituitary material from the adult *Necturus* have been shown to induce precocious metamorphosis in *Rana clamitans* larvae (Charipper and Corey, '30). This physiological test may be considered not alone as an indication of the potential value of the hypophysis, but also as evidence of cellular activity of its elements.

CONCLUSIONS

Confirmatory evidence is offered to show that the hypophysis of *Necturus maculosus* consists of four parts: pars anterior, pars intermedia, pars tuberalis, and pars nervosa. The pars tuberalis and the pars intermedia contain only basophilic cells. The pars tuberalis is non-vascular, while a few isolated capillaries may be found in the pars intermedia. The pars anterior has been shown to contain basophiles, eosinophiles, and 'chromophobic' cells. Osmiophilic substance is present in the cytoplasm of the 'chromophobe' and eosinophilic cells. This substance is similar to Golgi material and occurs in rings and large and small granules variously distributed in the different cells.

The eosinophiles have been subdivided, cytologically, into four varieties: cells with numerous small granules near the nucleus and grouped in the cytoplasm toward the capillary lumen; cells with numerous large granules almost filling the cytoplasm toward the free border; eosinophiles with a highly vacuolated cytoplasm; cells with many large spherical bodies described as 'secretory spheres.' These four types of cells have been linked together in a secretory cycle.

The occurrence of vacuoles, variations in osmiophilic substance, and in cytoplasmic granules and spheres have been interpreted as histological evidence of secretory activity in the pars anterior of the pituitary gland in *Necturus maculosus*.

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PLATE 1

EXPLANATION OF FIGURES

2 Transverse section through the hypophyseal complex, showing the position of the pars intermedia (*P.I.*) in relation to the pars nervosa (*P.N.*) and the pars anterior (*P.A.*). The cord-like arrangement of the cells in the pars anterior can also be seen. Helly's haematoxylin-eosin. $\times 100$.

3 One area from the field in figure 8 under higher magnification to show: A. An eosinophile with numerous large granules in the cytoplasm. B. An eosinophile with its cytoplasm highly vacuolated. Nassonov's; iron haematoxylin. Oil immersion.

4 Transverse section through the pars anterior, showing several eosinophiles in vertical section, bordering on a capillary and so oriented that their nucleus is farthest away from the blood sinus. Zenker-formic-osmic; Delafield's haematoxylin-eosin. $\times 440$.

5 Vertical section through an eosinophile cell, showing the presence of numerous small granules grouped near the nucleus in the cytoplasm toward the vascular lumen. Nassonov's; iron haematoxylin. Oil immersion.

6 The same as figure 7, but showing a smaller group of eosinophiles. Zenker-formic-osmic; Delafield's haematoxylin-eosin. $\times 440$.

7 Transverse section through the pars anterior, showing several eosinophiles with vacuolated cytoplasm as well as other eosinophiles containing numerous large granules. Nassonov's; iron haematoxylin. $\times 880$.

8 Vertical section through an eosinophile cell, showing the cytoplasm filled with numerous 'secretory' spheres. Nassonov's; iron haematoxylin. Oil immersion.

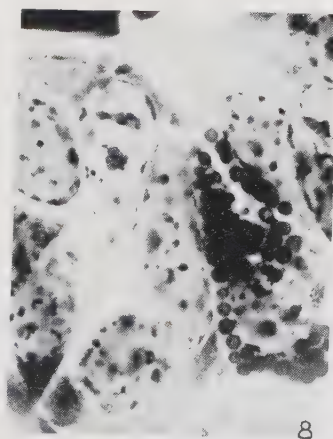
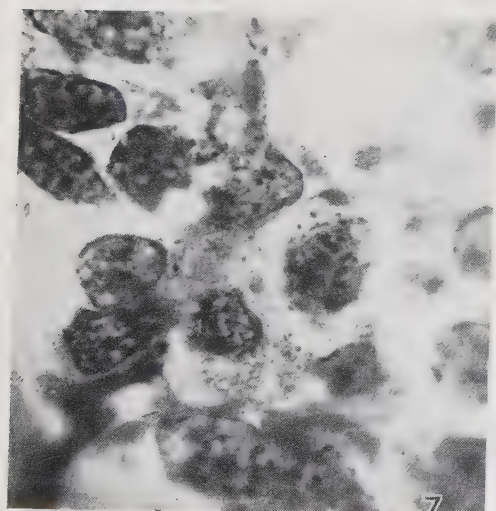
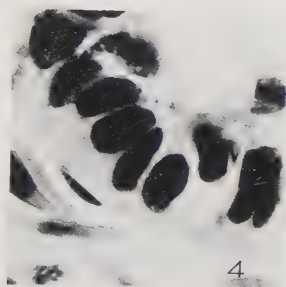
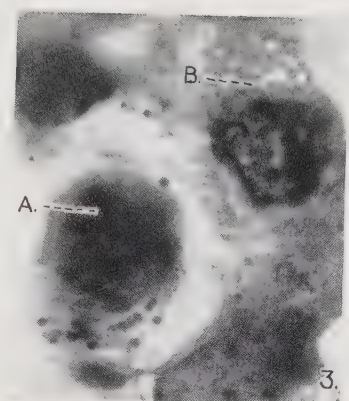
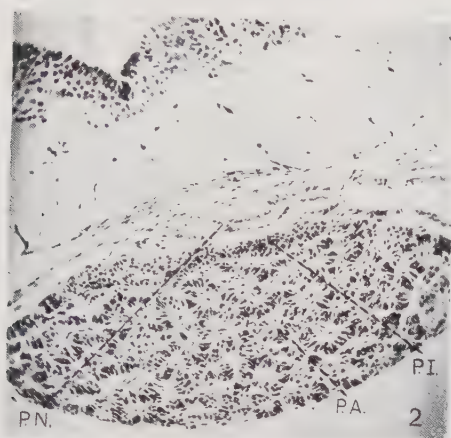


PLATE 2

EXPLANATION OF FIGURES

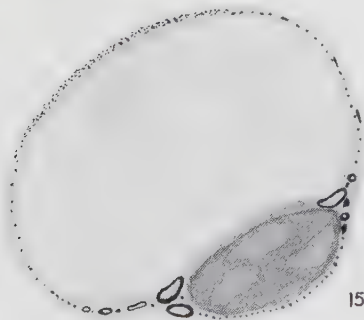
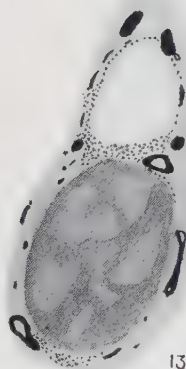
Camera-lucida drawings under oil immersion, showing cytoplasmic variations of chromophobic cells and eosinophiles of the pars anterior.

9 and 14 Two eosinophile cells taken from adjoining regions of the same microscopic field, showing variations in the amount and distribution of the osmiophilic substance. Mann-Kopsch; osmic acid. Oil immersion.

10 and 11 Two eosinophiles taken from adjoining regions of the same microscopic field, showing the variations in the cytoplasmic architecture. Nassonov's modification of Kolatchev's; osmic acid. Oil immersion.

12 A chromophobic cell showing the position of the osmiophilic rings and fine granules. Nassonov's modification of Kolatchev's; osmic acid. Oil immersion.

13 and 15 Two chromophobic cells taken from adjoining regions of the same microscopic field, showing variations in the size of the intracellular vacuole. Figure 13 shows primary and secondary condensation of granules about the nucleus. Mann-Kopsch; osmic acid. Oil immersion.



A NEW TYPE OF GRANULAR CELL IN THE ISLETS OF LANGERHANS OF MAN¹

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ONE COLOR PLATE (ONE FIGURE)

During the past thirty-five years the knowledge of the finer details of the structure of the islets of Langerhans has been increasing steadily. Laguesse ('95, '06) found that the islet cells were crowded with fine granules which could be seen in the living cells and which became very prominent when stained with safranin or gentian violet. Through the studies of Diamare ('99), Tschassownikow ('00), Schulze ('00), and Dewitt ('06), it was found that two types of granular cells are present in the islets. Lane ('07) showed that the granules of these two types of cells have markedly different solubilities.

As a result of these investigations, and especially those of Laguesse and Lane, it became quite clear that the granules in the islet cells were quite different from the zymogen granules in the pancreatic acinar cells.

The most extensive studies on the cells of the islets of the mammalian pancreas are by Bensley ('11, '15). He found that the granules in the islet cells could be stained supravitaly by Janus green or by neutral red and that the granules disintegrated when frozen. He was unable to distinguish two types of granular cells in his supravitaly stained preparations. Using a chrome-sublimate fixation, Bensley was able to stain the two types of cells (types A and B of Lane) differentially in the same preparation and to discriminate another type of cell (C) which was non-granular. With his

¹ This investigation was aided by a grant of the Rockefeller Foundation to the Biological Sciences Division of The University of Chicago.

neutral-gentian acid fuchsin method, Bensley found in the guinea-pig the A cells filled with red granules and the B cells with purple ones; the nuclei of the A cells were oval, while those of the B cells were round or slightly oval and contained large chromatin masses. He also found that all of the cells of the islets lacked the large eosinophil nucleolus such as is found in the acinar cells.

Bensley concluded that although the number of granules in the A cells varied in number and there was some indication of a development of non-granular cells into A cells, it was highly probable that the A and B cells were independent types. Bensley's conclusions have been confirmed by many authors (Opie, '28).

In 1924, Bowie reported the demonstration of three types of granular cells which could be stained differentially by a mixture of neutral ethyl violet and Bieberich scarlet in the islet tissue of the teleost, *Neomaenis griseus*.

Ukai ('26) found that with his modification of Mallory's aniline-blue method, the A cells of the rabbit islets stain red and the B cells, blue.

In sections of the human pancreas fixed in Zenker-formol and stained with the Mallory-azan method (Heidenhain's modification of Mallory's aniline-blue stain), I have found three types of granular cells in the islets of Langerhans. One of these is the A type, another the B type, and the third has not been described before in the mammalian islets of Langerhans.

The only reports of studies on the pancreas using the Mallory-azan stain that I have found are by Hett ('24), Neubert ('27), and Romeis ('29). Hett did not describe granules in the islet cells of the mouse pancreas in sections stained with the Mallory-azan method. Romeis recommends this method for demonstrating the reticular fibers in the gland. Neubert, in his extensive report on the development and structure of the human pancreas (from Heidenhain's laboratory), mentions (p. 106) that in the human embryonic pancreas two types of cells are to be seen in the developing

islets. They appear after the fourth or fifth month; one of the cell types was filled with deep red granules embedded in a yellowish cytoplasm. The remainder of the islet was made up of pale, grayish yellow cells. The granules in these cells, because of their delicate yellow color, were difficult to discriminate from the yellow cytoplasm. He found that the pale cells of the islets had larger nuclei, in general, than the deep red granular cells.

In the pancreas of adult men, Neubert found (p. 111) that the cytoplasm of the islet cells has a delicate bluish red or yellow color with the Mallory-azan stain. They contained many fine chromophobe gray granules which were especially prominent on those sides of the cells nearest the blood vessels. He concluded that these cells are of the B type described by Lane, Bensley, and Ukai. In the islets of the pancreas of the adult, Neubert was unable to find cells with very deeply staining granules such as he had found in human embryonic pancreases. He pointed out that these cells, which he believed were of the A type, have been demonstrated mainly in the rapidly growing pancreas of the lower mammals. In his figure 43 the cells of the islet appear homogeneously yellow and those of the acini reddish orange and without zymogen granules. His material was fixed in Heidenhain's 'Susa' mixture (sublimite, sodium chloride, water, trichloroacetic acid, glacial acetic acid, and formalin). This fixative probably accounts for the absence of granules in Neubert's sections in both acinar and islet cells as compared with the results obtained by the Mallory-azan stain after Zenker-formol fixation (see below and fig. 1).

MATERIAL AND METHOD

The following study is based on sections of four human pancreases. The material was obtained at necropsy on four individuals varying from six months to fifty-four years of age. In each case portions of the pancreas were fixed very shortly after death in Zenker-formol (nine parts of Zenker stock and one part of neutral formalin) for six to eight hours.

The shorter the interval between the death of the individual and fixation of the organ, the better the sections stained. The tissues were embedded in celloidin, serially sectioned by the method of Rubaschkin-Maximow, and stained after the celloidin had been dissolved from the slides by absolute ether and alcohol. In using the Mallory-azan stain, the procedure outlined in Romeis' histological manual was followed very carefully. One of these sections is now nearly four years old and seems not to have faded. This is probably due to the mounting medium, which consisted in the petroleum-ether extractive of crude damar, evaporated to dryness and dissolved in xylol.

OBSERVATIONS

As seen with the low power of the microscope, a section of the human pancreas prepared with the Mallory-azan stain shows the various tissues in the organ very distinctly. The collagenous and reticular framework is a brilliant ultramarine blue; the proximal portions of the pancreatic acinar cells are of a deep lilac color and the zymogen granules are a brilliant red, slightly tinged with orange; the islets of Langerhans are predominantly yellow orange, with a few pale blue and deep red areas in them. The yellowish islands thus stand out quite prominently against the surrounding purple and scarlet of the acini.

With the oil-immersion lens, the basal portion of the acinar cells stains various shades of lilac, purple, or purplish blue. This portion of the cell is quite homogeneous and 'smooth,' and contains in some acini a few rounded or slightly rod-like red dots. Some of these may be mitochondria; others, small zymogen granules. The nuclei of the acinar cells have large amounts of chromatin, and in most cases, but one large nucleolus, which stains a bright orange. The distal portion of the cells is usually crowded with dense masses of bright red—sometimes orange-tinged—zymogen granules. These extend to the lumen of the acini and seem to form almost continuous masses from one cell to its neighbors. Winding

about among these zymogen granules are clear, narrow, net-like areas which follow rather bizarre patterns. These are in all probability the canalicular apparatus. A suggestion of this appearance is indicated in several acinar cells in the accompanying figure.

In the sections of the pancreas obtained from a six months' infant dying of osteomyelitis and septicemia due to pneumococcus infection—this pancreas was fixed within fifteen minutes after death—the acinar cells are practically devoid of zymogen granules. In this instance the canalicular apparatus is as prominent as in sections specifically stained by the Golgi or Kopsch methods.

The centro-acinous cells and those of the ductal system are characterized by the orange-gray color of their cytoplasm and by their nuclei, which are smaller and more oval than those of the acinar cells.

The islets as seen under high magnification consist mainly of yellow-orange cells. These are filled with very minute, grayish orange granules which occupy most of the yellow cell body; in many of these cells are a few slightly larger, reddish granules. All of these orange-stained cells are B cells (fig. 1, B). Scattered throughout the islet, but usually arranged along its periphery or near the blood capillaries, are cells of another type. They contain closely packed, brilliantly red-stained granules. These are smaller than the red-stained zymogen granules, but definitely larger than the granules in the orange-gray cells just described and are of the A type (fig. 1, A). These granules may be dispersed throughout the cell bodies or may be confined to one part of the cytoplasm. When this occurs, it is usually that part of the cell which is nearest the blood vessels. In some of these cells, the part of the cytoplasm which is devoid of granules stains a very pale blue.

The third type of cell in the islets is stained blue by the Mallory-azan method (fig. 1, D). In most cases the pale blue-stained cytoplasm is filled with a mass of closely packed, bright blue granules which appear smaller than the red-

stained A granules. In some of the D cells, the cytoplasm appears homogeneously blue and it is not possible to see granules in them. Occasionally one of the 'blue' cells contains a sprinkling of bright dots similar in appearance to those in the A cells. It is difficult to say whether these are mitochondria or whether such cells are to be considered as transition forms between the D and A cells. Very rarely there is a cell which seems to contain both orange and blue granules. Here again it is difficult to decide whether these are transition forms from one type to the other or whether they are superposed, partially cut B and D cells.

In contrast to Bensley's findings in the islet cells of the guinea-pig, the islet cells of man frequently contain large, brilliant, orange-stained nucleoli. These are particularly prominent in the A cells.

In all of the islets the orange-gray-stained or B cells are the most numerous. The A and D cells are present, in most cases, in about equal numbers. There seems, however, to be no constant relationship in the numbers of the different cellular types. Some islets contain but few D cells, while others have but few A cells.

In sections of these same series of pancreases stained by the iron-hematoxylin method, three types of cells are to be made out. The majority of the cells are filled with very fine, blue-black dots and also contain a few larger, black granules. These are the B cells. The A cells are filled with coarser black granules. The third type of cell, which is very infrequent, is usually found at the periphery of the islets and does not contain any granules. These are very probably the C cells of Bensley.

I have searched very carefully in the Mallory-azan preparations for cells without granules. In some cases at the periphery of the islets are orange-stained cells with but very small amounts of cytoplasm in which it is difficult to determine whether granules are present or not. These are perhaps the non-granular C cells of Bensley, but it is certain that the Mallory-azan method is not useful in demonstrating them.

It seems best to designate the third type of granular cell in the islets of the human pancreas as D cells. Whether they are identical with the third type of granular cell which Bowie has found in the islet tissue of *Neomaenis griseus* requires further investigation. The D cells here described are in all probability not the same as Bensley's non-granular type-C' cell. The significance, both morphological and functional, of these blue-stained granules is unknown. They are, however, of constant presence in the several human pancreases examined until now with the Mallory-azan method.

SUMMARY

1. The Mallory-azan stain, when applied to sections of human pancreases fixed in Zenker-formol, demonstrates three types of granular cells in the islets of Langerhans.

2. One of these apparently is made visible for the first time in the mammalian pancreas. The significance of this new type of cell is not known.

3. When mounted in pure damar, one of these preparations has not faded for nearly four years.

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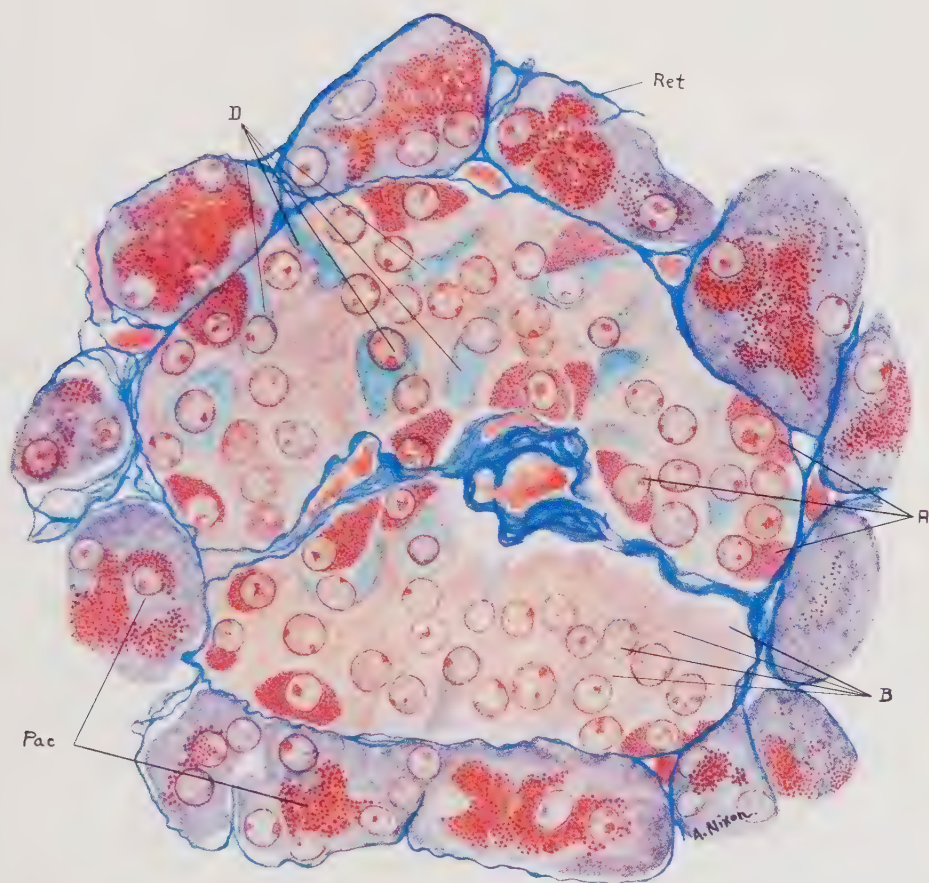
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PLATE 1

EXPLANATION OF FIGURE

1 Portion of a section of a human pancreas. The central part of the figure is an islet of Langerhans with granular cells of types A, B, and D. The islet is surrounded by pancreatic acini (*Pac*). The blue-stained reticular fibers are prominent (*Ret*). Zenker-formol fixation; celloidin embedding; Mallory-azan stain. Spencer 2-mm. apochromatic objective and 10 $\times$ ocular. The drawing was made with the camera lucida at table level and reduced $\frac{1}{3}$ in reproduction.



THE RELEASE OF FOLLICULAR COLLOID FROM THE THYROID OF AMBLYSTOMA JEFFERSONIANUM FOLLOWING HETEROPLASTIC ANTERIOR-PITUITARY IMPLANTS¹

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THREE TEXT FIGURES AND THREE COLOR PLATES (THIRTEEN FIGURES)

Experimental work has demonstrated that the thyroid of young *Necturus maculosus* may be stimulated to increased activity by anterior-pituitary implants. From a histological study of the thyroids of these experimental animals, it was concluded that the stored colloid was released into the blood capillaries through the cytoplasm of the follicular cells (Grant, '30 b). This demonstration of transcellular colloid export was especially convincing because the experimental studies could be arranged into a carefully timed series and the process of colloid release could therefore be observed from the initial step through complete emptying of the follicle.

Such an explanation of colloid release is directly opposed to the intercellular theory supported both by Uhlenhuth ('27) from his studies of the urodele gland and by Hirschlerowa ('27) from her observations upon the thyroids of the Anura. Both of these investigators based their conclusions, however, upon the study of thyroids during the process of normal metamorphosis. At this time the release of colloid is never so complete as that which can be obtained experimentally. The histological patterns are consequently much less extreme and therefore more difficult of interpretation than in the exaggerated patterns of the experimental series.

¹Contributions from the Department of Zoölogy, Smith College, no. 167. This study, aided by a Whitney Fellowship from Radcliffe College, was begun in the Agassiz Museum of Comparative Zoölogy.

The studies upon the *Necturus* thyroid appeared so convincing that it has seemed significant to make comparative experimental observations upon the thyroid of *Amblystoma jeffersonianum* and thus extend the analysis of the secretory mechanism of the urodele thyroid to a species which normally metamorphoses. Therefore the aim of the present study was to ascertain the effect of anterior-pituitary implants upon the larval thyroid of *Amblystoma jeffersonianum*. Experimental investigations concerning the mechanism of colloid release will thus have been extended to the thyroid of a species more typical of the urodele group than *Necturus* and more comparable to animals worked on by previous investigators.

The author wishes to acknowledge the continued interest and constructive criticism given to this study by Prof. Alden B. Dawson.

MATERIAL AND METHODS

The experiments were carried on during July and August of 1930 upon *Amblystoma jeffersonianum* larvae hatched in the laboratory from nests obtained in the field in early April. Experience showed this species to be the hardiest of the *Amblystoma* group. It developed rapidly and seemed to be immune to the fungus infection which caused high mortality rates in *punctatum* species which were being raised simultaneously. The animals were fed a mixed diet of *Daphnia* and *Enchytraeus*.

The individuals used for experimentation averaged 55 mm. in length, and although they were approaching the period of normal metamorphosis, only carefully selected, typical larval forms were used. In such animals there appeared no hint of blunting of the digits or atrophy of the dorsal fin—changes which in this species characterize the premetamorphic transformations (Grant, '30 a, '30 c).

The pituitary for implantation was obtained from male and female individuals of several species of frogs. The animal

was decapitated and the pituitary removed and placed into cold-blooded Ringer's solution. In this medium the anterior lobe was carefully separated from the other portions of the gland.

The *Amblystoma* host was anaesthetized in a 1:3000 chlore-tone solution. The frog pituitary was cut into two or three pieces and inserted into the peritoneal cavity. The incision was closed by one suture made of a single strand separated from grade-0 white silk. Various steps in the procedure were carried on under the dissecting microscope and parts of the technique were aided by the use of iridectomy scissors.

The tissues were fixed in Champy fluid and stained with Ehrlich's haematoxylin and Harrison's modification of Mallory's connective-tissue technique. The structures observed were seen only in material fixed in Champy. Zenker and Helly fixatives and modifications of these including Zenker with osmic acid failed to reveal clearly the details of cytoplasmic structure here reported.

This study is based upon the microscopic examination of the thyroids of twenty-one individuals which had received daily implants for one, two, three, four, five, six, or seven days and killed one day following the last implant. Figure 1 shows a photograph of ventral view of an individual which had received seven implants and the seven sutures are evident.

HISTOLOGY OF THE LARVAL GLAND

The thyroid of a larval *Amblystoma jeffersonianum* averaging 55 mm. in length consists of two bilateral masses each of which contains approximately from thirty to forty follicles. These vary considerably in size and the epithelium of the largest when seen in cross-section may contain as many as twenty-five cells. Figure 4 shows a longitudinal section of such a gland which reveals at this level about twenty follicles. The follicular cells are low, cuboidal in shape, and the vesicles are distended with abundant stored secretion. Figures 7 and 8 are camera-lucida studies of two follicles of different sizes

taken from glands of these larval animals and studied with apochromatic lens, oil-immersion and compensating oculars. These follicles are typical of those seen in the larval animals which served as controls for the experimental series. The



Fig.1 *Amblystoma jeffersonianum*. Ventral view. $\times 1.8$. Twenty-four hours after seven daily implants. Note seven sutures.

predominant low cuboidal cell showed no differentiation of the cytoplasm and no intracellular colloid. Upon careful search, occasional cells were found containing deep-staining colloid droplets or a non-staining vacuole. A very few of the smaller follicles contained one or two cells in which the cytoplasm, as in figure 8, appeared highly distended and chro-

mophobic. No interpretation of these enlarged, vacuolated cells is as yet possible except to state that such a picture is characteristic of the initial step in the process of colloid release. It should also be pointed out that these cells are not to be confused with the 'chief cells' first described by Langendorff ('89) and confirmed by subsequent workers.

The histology of the larval thyroid of *Amblystoma jeffersonianum* resembles in general the description given by Uhlenhuth for other urodeles during the period which he termed the 'developmental stage' at which time he believed the secretion was being stored. Champy fixation of these larval glands, however, shows occasional vacuolated cells and very rarely intracellular colloid dropets. These details have been previously reported by Charipper ('29) and Grant ('30 b) and interpreted by both workers as evidences of restricted regional activity. It seems likely, therefore, that in the so-called inactive gland the processes of storage and release of secretion are so nearly balanced that the morphological evidences of glandular activity are preserved by only the most delicate cytoplasmic fixations and then seen in very few cells.

HISTOLOGY OF THE THYROID FOLLOWING ANTERIOR- LOBE IMPLANTATION

Twenty-four hours after one implantation

The histological picture of the thyroid removed from an individual twenty-four hours after one implantation was not strikingly different from that seen in the larval, control gland. There appeared, however, a slight increase in the number of distended, non-staining, vacuolated cells and this change remained the only morphological evidence of any physiological change within the gland. This slight change appeared in contrast to the result found in the *Necturus* thyroid, which showed marked evidences of increase in activity within twelve hours after one implantation.

Twenty-four hours after two and three daily implants

An examination of the thyroids removed from animals twenty-four hours after two and three daily implants revealed only slight changes from the histology of the gland after one implantation. The number of distended, vacuolated cells was definitely increasing and in some cases intracellular colloid was abundant. Figure 10 shows a camera-lucida drawing of a follicle from a gland fixed twenty-four hours after two daily implants; and figure 9 is a similar study from a gland fixed twenty-four hours after three daily implants. These illustrations show several follicular cells which have become distended, changed in shape and in their staining reactions. In figure 10 numerous cells containing colloid droplets may be seen.

Although a very definite change in the physiological state of many of the follicular cells was thus evident, there appeared in no case any measurable release of stored secretion. This reaction again appeared in marked contrast to the response observed in the thyroid of *Necturus* which showed complete emptying of several follicles seventy-two hours after one implantation, whereas the thyroid of larval *Amblystoma jeffersonianum* stimulated at a time approaching normal metamorphosis failed to show any observable release of stored secretion twenty-four hours after three daily implants.

Twenty-four hours after four daily implants

All animals which had received four daily implants and were killed on the following day possessed thyroids which showed uniform and striking changes. Glands from these cases all showed a very definite and marked decrease in the amount of stored secretion and a simultaneous increase in the intracellular colloid content. Figure 11 shows a camera-lucida drawing of a follicle from such a gland. Figure 5 is a semidiagrammatic, camera-lucida, outline drawing of a longitudinal section from the thyroid of the same animal. This illustration is given to show that the particular follicle which has been selected for oil-immersion study and shown in fig-

ure 11 is characteristic of the follicles found throughout these glands. A section, therefore, taken at any level from such thyroids will show this predominating type of follicle.

An examination of figure 11 will show that the cells have become very tall and columnar in shape. The follicular colloid is reduced. The distended, non-staining, vacuolated cells have disappeared and there remain only small, non-staining vacuoles present in some cells. The intracellular colloid has increased and appears abundantly in every cell and in some cases resembles the form of an 'emulsion' of colloid in cytoplasm.

Twenty-four hours after five, six, and seven daily implants

Animals which had received five, six, or seven daily implants showed the great majority of the thyroid follicles to be practically empty of stored secretion. In fact, many were so nearly empty that when followed through in serial sections only traces of a lumen could be found. Figure 6 shows a semidiagrammatic, camera-lucida drawing of a section of a gland which was fixed twenty-four hours after six daily implants. This section shows several follicles which when followed through in serial cuts showed practically no lumen, and colloid material was seen chiefly within the cytoplasm of the cells which had become rounded into spheres or cord-like masses. Vesicle *a* in figure 6 is shown in detail in figure 12. There appear only occasional, small, non-staining vacuoles and every cell seems saturated with stainable colloid.

ACCELERATED AND MODIFIED METAMORPHOSIS
FOLLOWING IMPLANTATIONS

The incipient metamorphic period in *Amblystoma jeffersonianum* is marked by a thickening and blunting of the digits which is especially pronounced because they are unusually long and slender in the larva of this species. In this incipient period, which precedes the shedding of the first adult molt by six or seven days, there also appears the beginning of atrophy of the dorsal, tail fin. No alterations in pigmentation occur

during metamorphosis, so that the coloration differences, including bluish areas characteristic of the adult, must appear some time later.

Daily observations concerning morphological changes were made upon twenty-nine animals which received one, two, three, four, five, six, or seven daily implants. Twenty-four animals received at least three daily implants and none of these showed any definite premetamorphic changes on the day



Figs. 2 and 3 *Amblystoma jeffersonianum*; same animal as in figure 1. Figure 2 was taken on the day following two daily implants, at a time when no metamorphic changes were evident. Changes in this animal first began to appear on the day following the fourth implant. Figure 3 taken on the day following seventh implant. Metamorphic changes well advanced.

following the third implant. Nineteen individuals received at least four daily implants and fifteen of these showed premetamorphic changes on the day following the fourth implant. Of the remaining four, two individuals showed transformation changes on the day following the fifth implant and the other two were killed before they showed any metamorphic changes.

From a study of this series it is clear that the great majority of the animals showed no metamorphic changes

until the day following the fourth implant. Figure 2 is a photograph of an animal taken twenty-four hours after two daily implants, at a time when no premetamorphic changes were evident. Changes first began to appear in this animal on the day following the fourth implant, and figure 3 shows the same animal five days later, i.e., on the day following the seventh implant. At this time transformation changes were well advanced.

The animals which underwent precocious metamorphosis caused by these daily implantations of anterior pituitary showed several abnormal changes. They became very dark and abnormally emaciated and the atrophy of larval structures progressed rapidly. The initial shedding of skin was seldom cast off as in the characteristic cornified, adult molt, but remained attached and hung in small shreds over the entire body.

Three animals which received one single implant showed the first sign of metamorphic changes on the fourth day. These animals passed through the metamorphic transformations in a manner much more comparable to the normal process. The animals remained light in color and did not show the extreme emaciation of the other more accelerated experimental cases. The thyroids of these animals showed hyperactive glands resembling figures 9, 10, and 11. No vesicles were found which showed the completely empty follicles illustrated in figures 6 and 12.

DISCUSSION

From the foregoing description it is clear that anterior-lobe implants caused the release of colloid from the thyroid of larval *Amblystoma jeffersonianum*. Upon repeated stimulation, the majority of the follicles were practically emptied of the stored secretion.

The first evidence of this response was seen within twenty-four hours, when an increase in the number of distended, non-staining, vacuolated cells was evident. No release of stored secretion, however, could be observed at this time. Glands

studied on the day following two and three implants showed an increasing number of these vacuolated cells and in some cases intracellular colloid was fairly abundant. There still appeared no distinguishable reduction in the amount of stored secretion. A marked reaction in the thyroid was seen on the day following the fourth implant. In these glands the amount of follicular colloid was definitely reduced and the intracellular colloid, correspondingly increased. Further stimulation of the gland was brought about by five, six, and seven daily implants which caused the majority of the follicles to so release the stored secretion that the vesicles appeared with only a trace of a lumen and the cells were grouped into spherical masses. The cytoplasm of these cells appeared saturated with secretion material.

As a result of these experiments the theory of transcellular colloid release, previously presented from experimental work upon the thyroid of *Necturus*, is likewise given for the thyroid of larval *Amblystoma jeffersonianum*. The conclusion is based upon the facts that colloid was progressively released from the vesicles and that the persistent morphological pattern during this process was characterized by an abundant, intracellular colloid content. It does not seem possible, therefore, to interpret the colloid material within the cytoplasm of the follicular cells as new synthetic secretion products, but, on the contrary, it must be regarded as morphological proof of the transcellular method of colloid export.

The work of Ingram ('30) on the Golgi apparatus of the thyroid cells of *Rana clamitans* in relation to anterior-pituitary transplants is of interest in connection with the present report. Ingram stimulated the thyroids of these anuran larvae to increased activity by anterior-pituitary transplants ('in most cases two') made at an interval of a week. From studies of these thyroids removed 'nine days after transplantation,' he reported definite changes in the size and morphology of the Golgi apparatus, but no evidence of reversal of secretory polarity. It would now seem significant to study the rôle of the Golgi structure in a series of thyroids

which had been more completely timed and more actively stimulated.

Several workers have suggested that the 'colloid cell' of Bensley, the Langendorff cell, and the cell filled with non-staining vacuoles (Anderson vacuoles) represent cells in different physiological phases of the secretory cycle, rather than differently specialized epithelial structures. Such an interpretation, which assumes that all the follicular cells are physiologically undifferentiated, is supported by the present study, which shows the entire epithelium partaking equally in the process of colloid release.

The actual mechanism by which the cells take up the stored secretion and pass it through the cytoplasm into the blood sinusoids remains obscure. It is possible that some explanation of this process may be obtained when histological patterns demonstrating the refilling follicle are available. Experiments are now being planned which involve the feeding of thyroxin or the injection of inorganic iodine to animals in which the thyroid has been experimentally emptied of its stored secretion. It is hoped that such experiments will bring about a refilling of the gland and thus reveal in a carefully timed series the relation of the chromophobe to the chromophile secretion. Until such demonstrations are available, analyses of the secretion cycle and the mechanism of transportation of the colloid through the follicular cytoplasm must remain uncertain.

In many respects the process of colloid release from the urodele thyroid resembles the phenomenon of fat absorption by the intestinal epithelium. Experimental work has made it clear that fatty substances in the intestine are hydrolyzed by the fat-splitting enzyme of the pancreatic juices to form fatty acids and glycerol. Glycerol is absorbed directly. The alkalinity of the pancreatic juices saponifies the fatty acids to soluble soaps which are also readily absorbed. Within the intestinal epithelium there then occurs a resynthesis of these substances to form again the fatty substances, the presence of which may be demonstrated by the osmic-acid technique. The

histologist, however, has as yet found no method whereby the absorption of the soluble substances may be observed. The passages of the material into and out of the cell are therefore phases of a complex chemical reaction for which there appears no morphological evidence.

Numerous investigators, notably Schafer ('85), Reuter ('03), Mottram, Cramer, and Drew ('22), have studied the histology of the intracellular fat in the intestinal mucosa. Mottram and his coworkers studied fat absorption in relation to vitamins. They found that fat normally traverses the intestinal epithelium as fine droplets and often in the form of long streams. Animals fed on diets without proper vitamins showed an insufficient fat absorption and the 'absorption by streams' was not seen. Vitamins, therefore, apparently control the degree of emulsification of fat and therefore the rate of its absorption.

The absorption of colloid by the follicular cells likewise appeared within the follicular cytoplasm in the form of droplets (figs. 11 and 12) and often, in the more extreme reaction (fig. 12), in the form of 'streams.' From the previous work upon the thyroid of *Necturus* (Grant, '30 b) and from the present report upon the gland of *Amblystoma jeffersonianum* it seems that the phenomenon of colloid absorption by the follicular epithelium may be compared to the absorption of fat by the intestinal mucosa. However different the actual chemical reactions in the two phenomena may be, both processes fail to reveal any morphological expressions for the passage of the material into and out of the cells. In both reactions, it is only when the resynthesized material is in a free state within the cytoplasm that it appears visible.

Thyroid cells in the process of cell division during increased glandular activity have been previously reported. Numerous amitotic figures were observed in the thyroid of birds by Florentin and Weis ('30), and occasional mitotic stages were seen in the experimental studies of the *Necturus* thyroid (Grant, '30 b). In the later stages of increased colloid release there appeared in the thyroid of *Amblystoma jeffersonianum*

abundant mitotic figures. Figures 13, 14, 15, and 16 were all taken from the same gland as illustrated in figure 6. From a study of these figures it is evident that intracellular colloid is present during all phases of the mitotic process.

Pollister ('29) studied cell division in the pancreas of the dogfish, in order to make detailed observations upon a somatic cell during mitosis with special reference to the behavior of cytoplasmic components. It has often been suggested (Meves, '99; Champy, '13) that a cell during the process of proliferation reverts to its primitive, undifferentiated state; i.e., it becomes dedifferentiated. Saguchi ('20) reported no evidence of this return to the primitive condition in dividing pancreatic cells of the frog, since he observed no cessation of the secretory process in such cells. Pollister ('29), however, believed that the pancreatic cells of the dogfish gave evidence of a temporary cessation of the secretory process during cell division. He reported that the early stages in the development of secretory granules disappeared before the metaphase, and, although mature granules persisted throughout the course of the division, they were later destroyed and never secreted.

The dividing thyroid cells of the stimulated gland of *Amblystoma jeffersonianum* showed abundant intracellular colloid during all of the mitotic phases, and thus presented the same cytoplasmic patterns as other follicular cells. Therefore, as far as may be determined from histological evidence, there appears during mitosis no change in cytoplasmic activity of these follicular cells active in colloid release.

No experimental attempt has been made to explain the quantitative differences between the response of the thyroid of *Amblystoma jeffersonianum* and that described for the thyroid of *Necturus*. The fact that it required five or six daily implants to cause the complete release of colloid in the thyroid of *Amblystoma jeffersonianum*—a reaction which in *Necturus* was brought about in seventy-two hours following one implantation—may have various explanations. Two possible theories are suggested, however. The *Necturus* host was

10 mm. shorter and possessed a total body weight considerably less than the larval *Amblystoma*. The Necturus gland and the follicles were likewise much smaller and the stored secretion consequently less. The explanation may therefore be entirely expressed in terms of differences in relation of body size and thyroid size to the amount of implanted pituitary. That there is, however, in different species a definite variation in the degree of readiness of the thyroid to respond to pituitary implants was observed from a study of twelve *Amblystoma opacum* which had received one implantation. These animals showed at the end of two and three days a much more active thyroid than did the gland of *Amblystoma jeffersonianum* on the day following two and three daily implants. This theory of the differences in thyroid sensitivity of different species is also supported from the studies of the normal metamorphic process of these two types. *Amblystoma opacum* passed through the metamorphic process more rapidly and showed a much more active thyroid during the transformation period than was ever observed in the case of *Amblystoma jeffersonianum*.

These suggestions are based upon the assumption, which as yet has no experimental proof, that the anterior pituitary possesses the same potency for thyroid stimulation regardless of the seasonal changes which effect the reproductive system. The implanted pituitary used in the experiments upon *Necturus* and upon *Amblystoma jeffersonianum* were not all from the same anuran species, nor were the frogs all in the same physiological state in relation to the breeding season.

SUMMARY

1. Histological studies of the urodele thyroid during different stages of activity were made with Champy fixation and hematoxylin and Mallory's connective-tissue stains. The structures described were seen only in material fixed in Champy. Zenker and Helly fixatives and modifications of these including Zenker and osmic-acid solution failed to reveal the details of cytoplasmic structure here reported.

2. Thyroids of normal *Amblystoma jeffersonianum* larvae presenting no incipient metamorphic changes contained numerous follicles distended with stored secretion and lined with low, cuboidal cells. Occasional follicles contained a few cells which were distorted and columnar in shape and filled with large, non-staining vacuoles. Occasional colloid droplets were seen in both the vacuolated and the non-vacuolated cells.

3. Implants of frog anterior pituitary were made into the peritoneal cavities of similar larvae, and this report is based upon histological studies of twenty-one individuals which had received daily implants for one, two, three, four, five, six, and seven days and which were killed twenty-four hours following the last implant. The pattern of the gland from individuals which had received one, two, and three implants showed no marked changes from the normal picture except for slight but definite increases in the number of vacuolated cells. After four successive implants, however, the number of these cells had greatly increased, while a decrease in the size of the vacuoles was evident. A most striking change at this stage was the marked decrease in the amount of follicular colloid accompanied by a simultaneous increase in the amount of intracellular colloid. Following five or six daily implants, the follicles were practically emptied and all the thyroid cells were saturated with colloid, which appeared in the form of an 'emulsion' in the cytoplasm.

4. The data of these experiments support the theory of transcellular export of follicular colloid from the thyroid of a metamorphosing urodele. The same theory was previously advanced by the author from data obtained from similar experiments upon the thyroid of *Necturus maculosus*—a non-metamorphosing urodele.

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PLATES

EXPLANATION OF PLATES²

Figures 4 to 16 are camera-lucida drawings from the thyroid of *Amblystoma jeffersonianum*. Figures 4 to 6 are semidiagrammatic, outline drawings of longitudinal sections from the whole thyroid, magnified approximately $\times 20$. Figures 7 to 12 are follicles in cross-section which have been magnified approximately $\times 900$. Details were added free-hand from oil-immersion study. Figures 13 to 16 are separate cells magnified approximately $\times 1500$ and the details were also added free-hand from oil-immersion study. All illustrations show staining reactions similar to those obtained with Champy fixation, haematoxylin and Harrison's modification of Mallory's connective-tissue technique. The stainable colloid, wherever it appears, has been shown in blue.

² The author acknowledges a grant from Smith College and also one from Radcliffe College which made possible the publication of the colored plates.

PLATE 1

EXPLANATION OF FIGURES

- 4 Normal, control animal. Longitudinal section of whole gland.
- 5 Twenty-four hours after four daily implants of anterior pituitary. Longitudinal section of whole gland.
- 6 Twenty-four hours after six daily implants of anterior pituitary. Longitudinal section of whole gland.

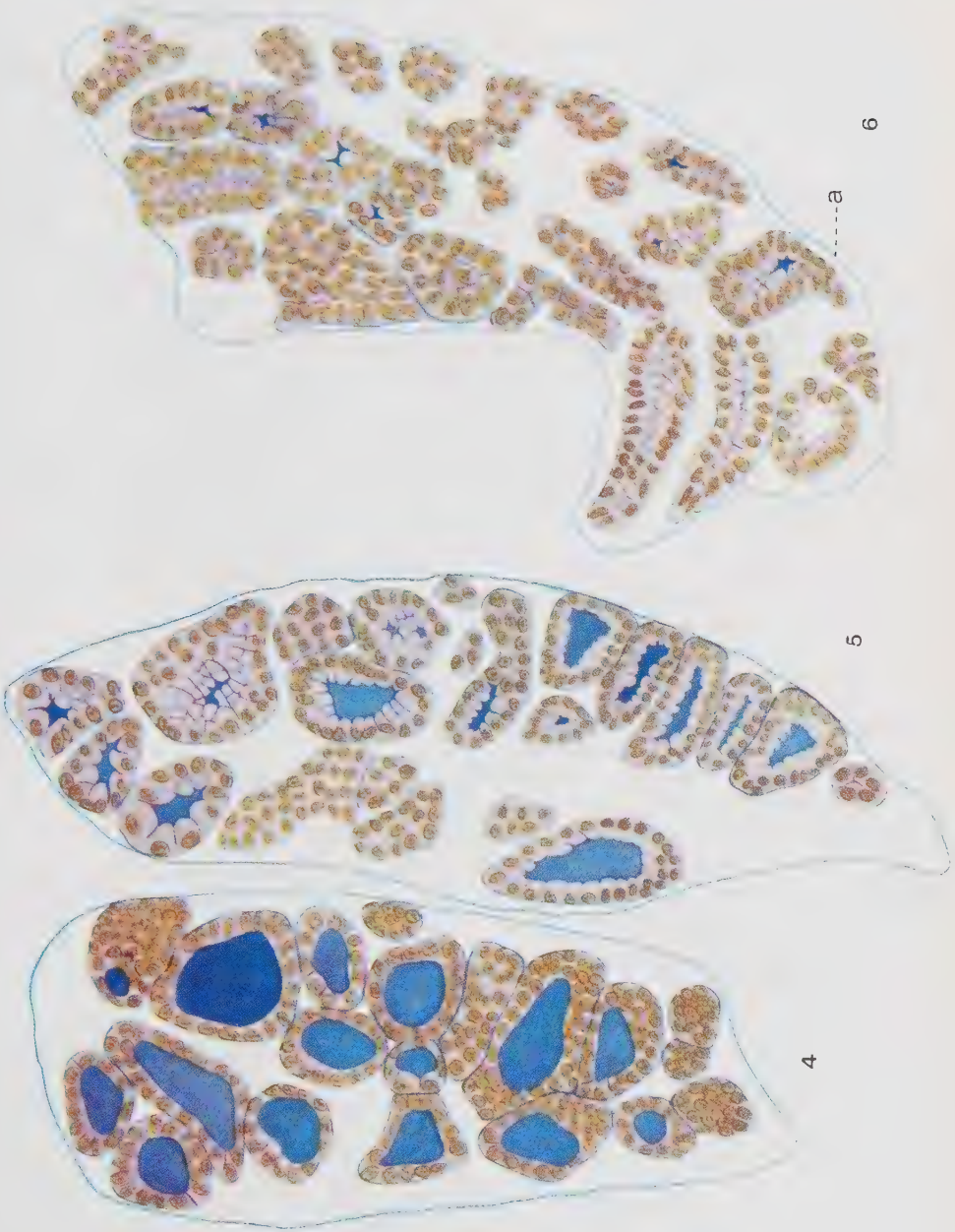


PLATE 2

EXPLANATION OF FIGURES

7 and 8 Normal, control animal. Cross-section of single follicle.

9 Twenty-four hours after three daily implants of anterior pituitary. Cross-section of single follicle.

10 Twenty-four hours after two daily implants of anterior pituitary. Cross-section of single follicle.

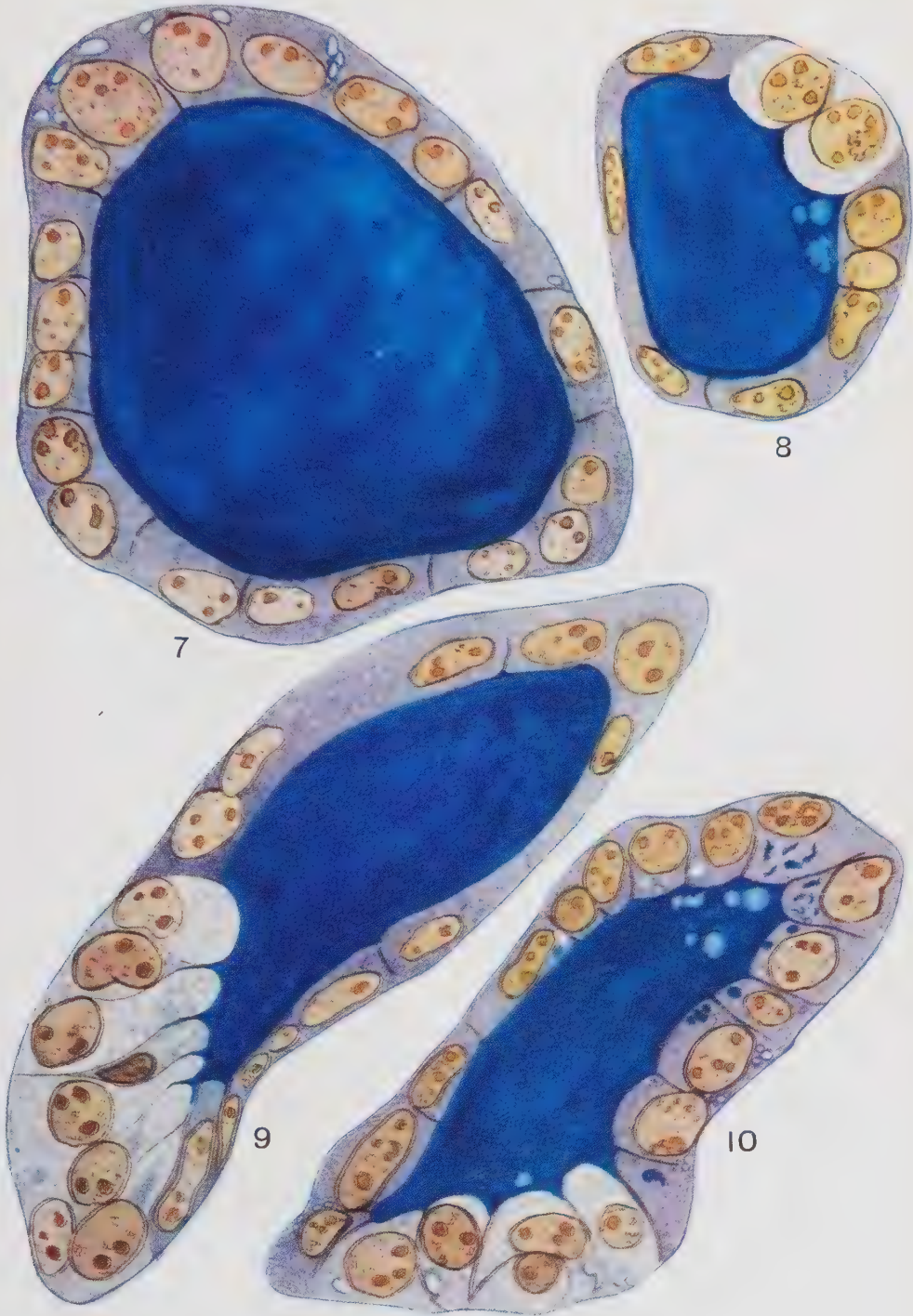


PLATE 3

EXPLANATION OF FIGURES

11 Twenty-four hours after four daily implants of anterior pituitary. Cross-section of follicle from same animal from which figure 5 was taken.

12 Twenty-four hours after six daily implants of anterior pituitary. Cross-section of follicle *a* seen in figure 6.

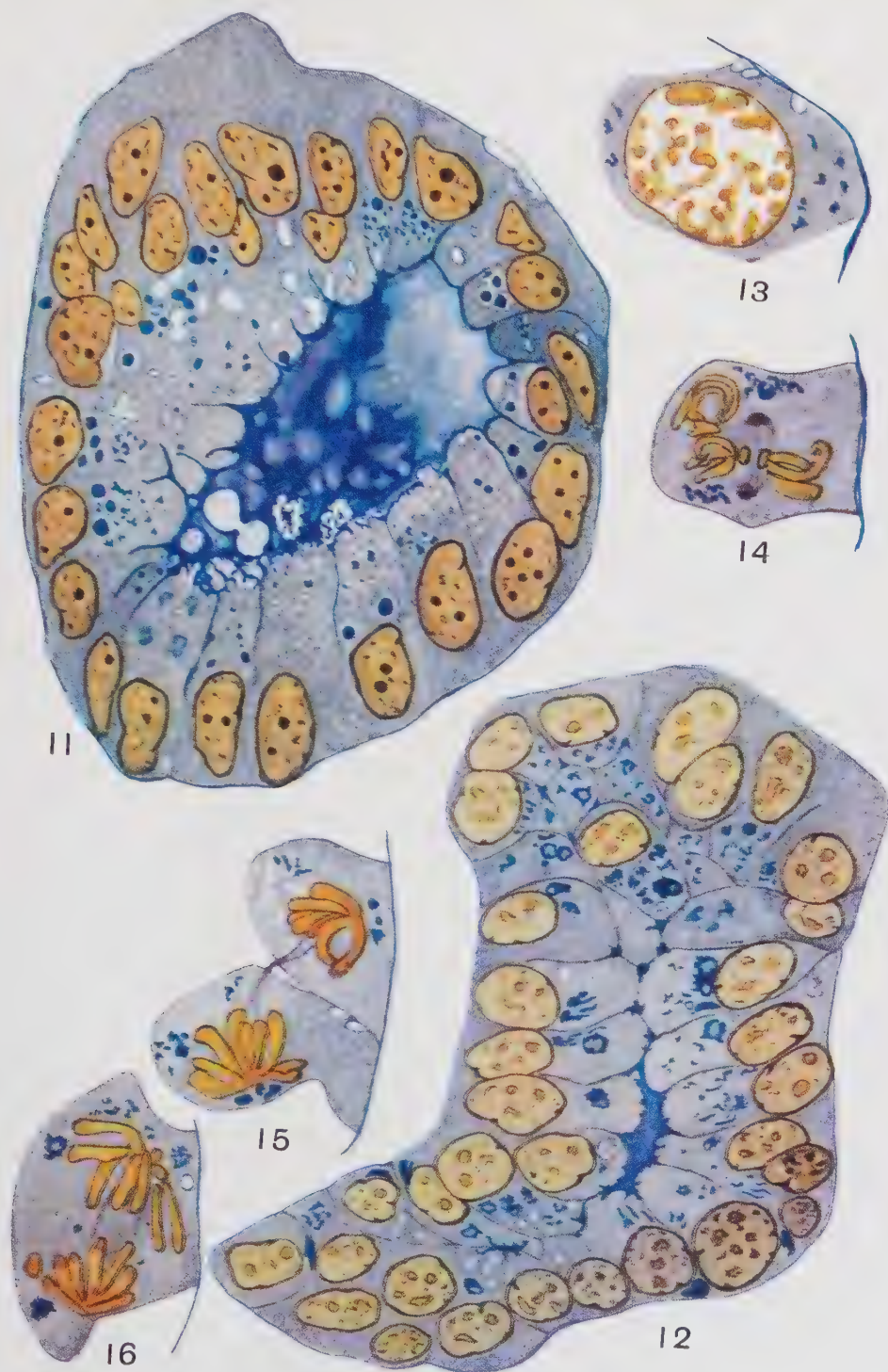
13 to 16 Mitotic figures taken from various follicles from the same gland illustrated in figure 6. The so-called basement membrane is shown in blue and appears at the right of each cell. Note that the plane of cleavage is in each case at right angles to a tangent drawn to the outer circumference of the follicle.

13 Prophase.

14 Metaphase.

15 Telophase.

16 Anaphase.



MITOCHONDRIA IN OSTEOCLASTS

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ONE PLATE (FOUR FIGURES)

It is well known that the multinucleate cells called osteoclasts which are found in the sections of developing bone are commonly regarded as the direct agents of bone resorption. Jordan ('18), Jackson ('04), Danchakoff ('09), and Maximow ('10) state that osteoclasts are produced from mesenchymal or reticular cells by a process of fusion in the early stages of bone development. Arey ('20) thinks that osteoblasts give rise to osteoclasts and also describes to some extent their fate, but the real function of these cells is still obscure at the present time.

METHOD AND MATERIAL

Decalcification is necessary for cutting bone tissue, but after decalcification the cytoplasmic components of cells are largely destroyed. The upper and lower jaws of a hamster two days after birth and the stripped periosteum of a newborn goat were used for the following experiments. The tissue was fixed in Regaud's fluid, embedded in paraffin, stained with Altmann's anilin-acid fuchsin, and counterstained with methyl green. Owing to the softness and delicacy of the membranous bone of the dental alveoli, sections were obtained without decalcification.

OBSERVATIONS

In recent years, valuable contributions have been made showing that mitochondria may be presecretion material. Bensley, Cowdry, Ma, and others have investigated this

matter. Most cells when functional contain more mitochondria, while, in degenerating cells, the mitochondria are decreased and become obscure or disappear. Our experiments show that osteoclasts often contain abundant mitochondria. At the time when the mesenchymal cells are changing their form and fusing into osteoclasts, mitochondria gradually increase in number (fig. 1). The adjacent mesenchymal cells contain fewer mitochondria and retain their original shape and size. Osteoblasts also show the same phenomena. As the osteoblasts join with the osteoclasts, the mitochondria increase in the cytoplasm of the cell (fig. 2). These osteoblasts, which are very close to osteoclasts, but are still functioning as bone producers, show no change in mitochondrial content. Though old osteoclasts are multinucleated, our observation shows that they are mononucleated when they are young cells (fig. 3). The reticular cells of the marrow show that the osteoblasts (fig. 2) may become mononucleated osteoclasts by increasing their mitochondria and by growing larger in size. They are quite different from other osteoblasts or reticular cells, for they contain proportionately more mitochondria.

DISCUSSION

Arey ('20), in the summary of his paper, states:

Osteoclasts undergo retrograde changes and ultimately disappear through extreme degeneration. Only indirect and insufficient evidence points to osteoclasts as the active, specific agents of bone resorption. That they are merely degenerating fused osteoblasts agrees better with the known facts.

If the osteoclasts are degenerating cells, why are their mitochondria increased? Why do the mesenchymal cells or osteoblasts increase their mitochondria and become changed into osteoclasts before degeneration? They are often closely applied to the resorption surface of the bone and, though we cannot prove by direct evidence that they are certainly bone destroyers, yet it seems to us that they are certainly not degenerating cells. Vacuolated cytoplasm is often seen in

osteoclasts, but this does not necessarily show degeneration, as their nuclei are still apparently in normal condition and there is no change in their mitochondria (fig. 3). Further study is necessary in order to state clearly the real function of osteoclasts.

SUMMARY

1. The mitochondria of the mesenchymal cells, reticular cells, or osteoblasts are increased as these cells become osteoclasts.

2. Osteoclasts contain more mitochondria than any other cells in the area where bone is developing.

3. Osteoclasts may be either multinucleate or mononucleate.

4. Osteoclasts are apparently not degenerating cells, even though their cytoplasm contains vacuoles.

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PLATE 1

EXPLANATION OF FIGURES

Tissues fixed in Regaud's fluid, stained with Altmann's anilin-acid fuchsin, and counterstained with methyl green. (Camera-lucida drawing.)

1 Showing early stage of osteoclasts developing from mesenchymal cells in which the mitochondria are increased. Young osteoclasts, one with a single nucleus, the other, two nuclei, *Oct'*; a large osteoclast with five nuclei and one vacuole, *Oct.* $\times 540$.

2 Illustrating osteoblasts connecting and fusing to become osteoclasts; mitochondria increased. Young osteoclast, *Oct'*, connected with an advancing osteoclast, *Oct.*; bone cell with mitochondria, *B.C.* $\times 630$.

3 Showing osteoclasts, both young and old, which contain more mitochondria than any other cells. Mitochondria in vacuolated osteoclast not diminished. A young mononucleate osteoclast, *Oct''*; binucleate osteoclast, *Oct'*; and a vacuolated osteoclast with eight vacuoles and seven nuclei. $\times 630$.

4 A large osteoclast with six nuclei obtained from the tibial periosteum of a newborn goat. $\times 630$.

ABBREVIATIONS

B.C., bone cell
M.C., mesenchymal cell
Mtx., bone matrix
Obt., osteoblast

Oct., *Oct'*, *Oct''*, osteoclasts
Pre.mlc., premyelocyte
R.C., reticular cell
R.B.C., red cell



NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

BREEDING AND CARE OF THE ALBINO RAT FOR RESEARCH PURPOSES, by Milton J. Greenman and F. Louise Duhring. Second edition. 1931. Size, $6\frac{1}{2} \times 10$ inches. Bibliography. Illustrated. Fabrikoid. Price, \$3.00. The Wistar Institute Press, Philadelphia.

Investigators, technicians, and others whose work is concerned with albino rats will be glad to know that the second edition of this book, revised and enlarged, has been prepared. It follows closely the lines of the first edition. Some sections have been rewritten to include additional growth records of the well-known Wistar Institute strain of albino rats. The procedures used successfully in producing and maintaining this standard research animal are described and explained. It contains information on the natural history of the rat, tested methods of breeding, housing, feeding, and exercising albino rats for the production of vigorous, fertile stock, and practical suggestions for their protection against diseases and parasites. Details of diet, cage construction, and colony equipment are fully presented. Variations in growth may be largely eliminated by following the routine suggested in this book.

DIE REFLEKTORISCHE SELBSTSTEURERUNG DES KREISLAUFES, by Eberhard Koch. (Ergebnisse der Kreislaufforschung, herausgegeben von Bruno Kisch, Band I.) 1931. Size, $6\frac{1}{2} \times 9\frac{1}{2}$ inches. Pages x + 234. Subject and author indices. Bibliography. 44 illustrations. Price, RM 18; bound, RM 19.50. Theodor Steinkopff, Dresden-Blasewitz.

This book, dealing with the reflex adjustment and regulation of the circulatory system, is the first volume of a series presenting all phases of research on circulation. It discusses the morphology, physiology, and pathology of the nerves supplying the organs of circulation, the functional relation between sinus pressure and the reflex responses of heart and blood vessels, and the interaction of their nerve centers.

ARBEITEN AUS DER HIRNFORSCHUNGSABTEILUNG (Vorstand Prof. C. v. Economo) des Laboratoriums der psychiatrischen Klinik in Wien.

This volume, representing the work of the laboratories of the Psychiatric Clinic in Vienna, is made up of the following reprints: Wie sollen wir Elitegehirne verarbeiten? by C. v. Economo, 87 pages, 22 illustrations; Zur Frage des Vorkommens der Affenspalte beim Menschen im Lichte der Cytoarchitektonik, by C. v. Economo, 113 pages, 22 illustrations; Cytoarchitektonik der Grosshirnrinde eines 5 jährigen und eines 1 jährigen Kindes, by José Aldama, with comments by C. v. Economo, 109 pages, 53 illustrations; Morphologische und cytoarchitek-

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tonische Studien über den Bau der unteren Frontalwindung bei Normalen und Taubstummen, ihre individuellen und Seitenunterschiede, by Erwin Stengel, 47 pages, 10 illustrations; Über Windungsrelief, Masse und Rindenarchitektonik der Supratemporalfläche, ihre individuellen und ihre Seitenunterschiede, by C. v. Economo and L. Horn, 80 pages, 16 illustrations; Die Supratemporalflächen eines Taubstummengehirnes, by Ludwig Horn, 17 pages, 9 illustrations; Beitrag zur Cytoarchitektonik des Operculum Rolando, by C. v. Economo, 8 pages, 2 illustrations; Nochmals zur Frage der arealen Grenzen in der Hirnrinde (Antwort auf die Vogtschen Darstellungen), by C. v. Economo, 8 pages; Cytoarchitectony and progressive Cerebration, by Constantin von Economo, 9 pages, 18 illustrations.

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NOTICES BY F. E. WELLS

THE DIAGNOSIS AND TREATMENT OF BRAIN TUMORS, by Ernest Sachs. 1931. Size, $6\frac{1}{2} \times 9\frac{1}{2}$ inches. 396 pages. Illustrated with 217 figures and six colored plates. Index. Cloth. Price, \$10.00. The C. V. Mosby Company, St. Louis.

"Brain tumors are not rare diseases," writes the author of this book. "They occur so frequently that every physician may expect to encounter them and should know how to recognize them." Hitherto there has been no book which gave the subject the thorough treatment deserved, but in this volume Doctor Sachs has brought together from his vast knowledge and experience the facts essential to making a localizing diagnosis. The first chapter treats of the surgical anatomy and physiology of the brain from the viewpoint of the neurosurgeon. The next six chapters show the steps by which the diagnosis of a case of brain tumor is reached, and the eighth chapter mentions the principal diseases apt to be confused with brain tumors and how they may be differentiated. The final chapter gives details of operative technique and postoperative care.

ANIMAL MOTIVATION—EXPERIMENTAL STUDIES ON THE ALBINO RAT, by C. J. Warden, with the collaboration of T. N. Jenkins, L. H. Warner, Marion Jenkins, E. L. Hamilton, and H. W. Nissen. 1931. Size, 6×9 inches. Pages xii + 502. Illustrated with charts, tables, and drawings. Index. Bibliographies. Cloth. Price, \$5.00. Columbia University Press, New York.

Reports on a long series of experiments in a study of the primary determiners of animal activity. The animals used were albino rats from The Wistar Institute Colony, and they were tested by the Columbia obstruction method to determine the force of the hunger drive, the thirst drive, the sex drive, the maternal drive, and the exploratory drive. Part I gives a complete description of the obstruction method of testing and compares it to other methods employed by investigators. Parts II to VI discuss the five drives and part VII compares their relative strength and persistence. Four papers included as appendices are studies of motivation tested by methods other than the Columbia obstruction method.

EMBRYOLOGY OF THE PIG, by Bradley M. Patten. Second edition. 1931. Size, $6 \times 9\frac{1}{4}$ inches. Pages ix + 327. 169 illustrations, some in color. Index. Bibliography. Cloth. Price, \$3.50. P. Blakiston's Son & Co., Inc., Philadelphia, Pa.

The second edition, like the first, presents the essentials of mammalian development with clearness and simplicity. Only minor revisions have been made in the text, except in those sections dealing with foetal circulation and with changes in

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the circulation following birth. These chapters have been extensively altered to conform with recent researches and discoveries which have necessitated revision or abandonment of previously accepted interpretations. The pig embryo is used as study material because of its value and availability for laboratory work; however, the book stresses the general principles of mammalian embryology rather than details peculiar to the pig.

REFLEX ACTION, A Study in the History of Physiological Psychology, by Franklin Fearing. 1930. Size, 6×9 inches. Pages xxiv + 350. Bibliography. Subject and name indices. Illustrated. Cloth. Price, \$6.50. The Williams & Wilkins Company, Baltimore.

This history of the development of the reflex-arc concept includes an account of the discoveries of the phenomena which the theory seeks to explain. It is divided into five periods: 1) the prescientific period, 2) the speculation period, 3) the period of nascent experimentation, 4) the period of the development of knowledge regarding the structural components of the reflex arc, and, 5) the modern period. The periods are taken up chronologically, chapters I to XI covering the seventeenth, eighteenth, and nineteenth centuries, and the last chapters (XII to XVIII) dealing with current problems. With a masterly selection of material, the author has confined his book mainly to phases of the subject which are related directly to reflex action as an explanatory principle in physiological psychology.

But such are the ramifications of the subject that it will appeal not only to physiologists, but to workers in the fields of anatomy, psychology, and philosophy.

BULLETINS ET MÉMOIRES DE LA SOCIÉTÉ DES CHIRURGIENS DE PARIS, Tome XXIII, nos. 1 and 2, meetings of January 9th and 23rd. Tome XXIV, no. 3, meeting of February 6th.

These publications contain the papers and lectures given at the Society's meetings. A supplement accompanying each issue supplies brief abstracts in six languages of all articles presented.

CONCERNING EARLIEST FIRST GROWTH IN THE HUMAN OVUM: Origin of First Blood Corpuscle and Plasm; First Blood Space and Vessel; Blood-corpuscle Differentiation from the First Multinucleolated Uncolored Nucleus; Blood Corpuscle through the Red Mononucleated Erythroblast to the Mature (Seventh to Eighth Week) Non-nucleated Red Erythrocytes; The Blood Placid of Minot; Theory for Origin of Tumors; Dermoid and Ovarian Cysts and of the Cancer, by Frank A. Stahl. 1931. Size, $6 \times 9\frac{1}{4}$ inches. 157 pages. 93 illustrations. Personally published by the author, Chicago.

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ANIMAL AGGREGATIONS, by W. C. Allee. 1931. Size, $6\frac{1}{4} \times 9$ inches. Pages $x + 431$. Illustrated with figures, charts, and tables. Index. Bibliography. Cloth. Price, \$5.00. University of Chicago Press, Chicago.

In the term 'animal aggregations' are included all those loosely integrated collections of animals not well enough organized to be called social colonies. They are considered with regard to their ecological and behavioristic physiology and with regard to their strictly social implications. As an introduction, the general background is sketched, a classification of aggregations is made, and some discussion is given on their formation, conditioning factors, and integration. The main body of the book considers the effects, both good and bad, of aggregations. The harmful effects include reduced growth, retarded reproduction, and increased death rate. The beneficial effects take in stimulation of growth, stimulation of reproduction, protection from toxic reagents, communal activity of bacteria, mass physiology of spermatozoa, and other problems. Morphological changes and determination of sex are classed as general effects. Chapters on animal aggregations and social life and the principle of cooperation conclude the book.

DAS POLLERSCHE VERFAHREN ZUM ABFORMEN AN LEBENDEN UND TOTEN SOWIE AN GEGENSTÄNDEN. Anleitung für Mediziner, Anthropologen, Kriminalisten, Museumspräparatoren, Prähistoriker, Künstler, Handfertigkeitslehrer, Amateure, by Alphons Poller. Edited by E. B. Poller and E. Fetscher. Foreword by C. v. Economo. 1931. Size, 7×10 inches. Pages $xii + 216$. 129 illustrations. Index. Price, RM 12; bound, RM 14. Urban & Schwarzenberg, Berlin and Vienna.

A description of the process worked out by Doctor Poller for making models of living and dead specimens and other objects. Dr. C. v. Economo writes in the foreword that the Poller method compares with the old plaster-of-Paris-cast method as a camera-lucida drawing compares with a daguerreotype.

THE VITAMINS, by H. C. Sherman and S. L. Smith. 1931. American Chemical Society Monograph no. 6. Size, $6 \times 9\frac{1}{4}$ inches. 575 pages. Illustrated. Subject and author indices. Extensive bibliography. Cloth. Price, \$6.00. The Chemical Catalogue Company, New York.

A summary of the known facts about those widely differing substances classed together as vitamins. It reviews the information gained regarding their chemical nature and the far greater knowledge of their rôles in life processes, their formation and distribution in nature, their relative abundance in different types of

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food materials, and their stability under conditions to which they are likely to be subjected. A separate chapter deals with each of the vitamins—B (B_1), G (B_2), C, A, D, and E. The extensive bibliography of 133 pages makes the book doubly useful. It contains the literary references available up to the middle of the year 1930. The appeal of the book is not to the professional chemist only, since its subject matter touches also the research fields of physiology, pathology, nutrition, and health.

THE PHYSICAL BASIS OF PERSONALITY, by Charles R. Stockard. 1931. Size, $6 \times 8\frac{1}{2}$ inches. 320 pages. 74 illustrations. Bibliography. Index. Cloth. Price, \$3.50. W. W. Norton & Co., Inc., New York.

The author writes, in a style popular rather than technical, of the physical factors underlying personality. He devotes several chapters to the genes as the first determiners of personality, to the developmental or embryonic personality, and to the mutations and character alterations of earliest life. From its origin he traces the growth of the individual through the processes and reactions of development to the finished person. Man differs from all other animals in having a relatively large brain and an exaggerated period of immaturity, both of which increase his learning period and his adaptability to environment. In the course of experiments on growth and type in the breeds of dogs, Doctor Stockard has made significant discoveries, some of which are applicable to the human race and shed light on the rôles played by the endocrine secretions and by brain shape in the production of the extremes of variation found in human races.

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NOTICES BY F. E. WELLS

AN INTRODUCTION TO NEUROLOGY, by C. Judson Herrick. New. Fifth edition. Cloth. Price, \$3.00. 417 pages with 140 illustrations. W. B. Saunders Company, Philadelphia.

The demand for Professor Herrick's "Introduction" has required ten printings and four revisions in the fifteen years since it first appeared. In this edition three chapters have been entirely rewritten, namely, those on the neuron, on reflex circuits, and on the general physiology of the nervous system. All chapters have been revised in the light of increasing knowledge. While really and intentionally an introduction leading to a more detailed account of morphology, it has proved itself an admirable text-book. The fundamental principles of nervous structure and function are set forth with a clearness and soundness which make it a most satisfactory text-book for students in psychology, education, and sociology. And because of Professor Herrick's deep insight into the evolutionary development of 'the master tissue' and of the clearness with which the nature of its different parts is presented in this book, it will continue to be of great service to students of anatomy and medicine.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN, Lieferung 355. Edited by Emil Abderhalden. Abt. V. Methoden zum Studium der Funktionen der einzelnen Organe des tierischen Organismus, Teil 2, Heft 16. 1931. Size, 7 × 10 inches. Pages 1815 to 1924. Price, RM 6.50. Urban und Schwarzenberg, Berlin and Vienna.

This issue contains three articles as follows: Die Mikroveraschung als histochemische Hilfsmethode, by A. Policard and H. Okkels; Nachweis und Bestimmung der Thymonukleinsäure, by Zacharias Dische; and Dichroitische Färbung tierischer und pflanzlicher Gewebe, by W. J. Schmidt. The third paper is illustrated with twenty-four text figures and one colored plate.

Received for Reviewing

(Notices will appear in a later number)

ARBEITEN AUS DEM NEUROLOGISCHEN INSTITUTE, Bd. 33, Heft 1. Franz Deutricke, Leipzig und Wien.

TOLDT, ANATOMISCHER ATLAS, 15th ed., Bd. 1, 2, 3. Urban und Schwarzenberg, Berlin und Wien.

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